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Aspects of arbuscular mycorrhizal (AM) fungal ecology

AM fungal nutrient-function efficiency in a primary sand-dune ecosystem on the west coast of India

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ASPECTS OF ARBUSCULAR MYCORRHIZAL (AM) FUNGAL ECOLOGY: AM FUNGAL NUTRIENT-FUNCTION EFFICIENCY IN A PRIMARY SAND-DUNE ECOSYSTEM ON THE WEST COAST OF INDIA

By

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It is as normal for the roots of plants to be mycorrhizal as it is for the leaves to photosynthesize.

Dr. Barbara Mosse

***frontis*:** trunks of a senescing 'arbuscule' and adjacent AM fungal hyphae.

Abstract

Arbuscular mycorrhizal (AM) fungi are root and soil inhabiting symbionts with higher plants. The fungi are especially nutrient-function efficient in nutrient deficient soils. There have been innumerable studies of AM fungal facilitation of plant nutrient uptake in controlled environments. Comparatively little similar investigation has been undertaken in natural soils, including investigation of taxon specific nutrient-function efficiency in the phylum.

Plant diversity and frequency, soil chemistry statuses, and AM spore diversity and abundance were sampled in an interrupted-belt transect in an aggrading dune sytem on west-coast India, followed by foredunes and transect nutrient amendment experiments in selected plant species.

The transect extends 175 m inland from mean high-water mark (MH-WM). Examination showed nutrients were consistently deficient. A plant zonation pattern and increasing frequency over the transect were indicated, as well as decreasing pH and increasing organic matter (OM)-amendment AM species diversity gradients. Plant zonation does not correlate with soil chemistry. There was a distinct soil transition at the 175 m point and evidence of further system partition between foredune and behind-foredune regions.

Plant and AM demographies bore no resemblance suggesting neither is driven by the other. Four AM genera were recovered, *Acaulospora*, *Gigaspora* and *Scutellospora* in high abundance, *Glomus* in comparatively low abundance. The two co-dominant species, *A. spinosa* and *Gi. margarita*, displayed divergent strategies in OM amendment. Certain AM taxa may be functionally associated with particular soil nutrients. There was no evidence of taxon-specific nutrient-function efficiency.

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Especial thanks go to my wife Elena who, despite my long periods of preoccupation, has consistently remained encouraging throughout the project. Spaciba.

CHAPTER 1

INTRODUCTION and LITERATURE REVIEW

1.1. Introduction

Soil is the most complex ecological sphere of all, "… perhaps the most intricate biological cycle in the world" (Brady 1974), a shallow living structure overlaying base landform from which green plants rise to interact with atmosphere and solar energy. Its structural, chemical and biotic integrity is vital to the maintenance of a stable global ecosystem, and loss of food-production land (Govt. of India 1st Five Year Plan 2007, Turbé *et al.* 2010, U.S. Environmental Protection Agency 2012) and natural environment (Flynn *et al*. 2009, International Union for Conservation of Nature n/d) are matters of increasing concern. Whether in conservation, restoration or sustainable agriculture, there is a need for greater understanding and knowledge of interactions between soil chemistry characteristics, climatic environment and biotic trophic levels drawn from evidence in ecosystems.

Coastal sand dunes are at the 'beginnings' of soil, a flexible barrier to the encroaching sea, possibly one of the terrestial environments into which plants first invaded. They are the point at which cycled psammite material is returned to the shore affording opportunity for soil genesis through plant stabilization and succession over space and time (Olson 1958, Ranwell 1960, Walker *et al*. 2010). Globally, in primary coastal sand-dune systems, soil nutrient deficiencies, high levels of salinity and aeolin sand-particle seedling burial contribute to an environmental inhospitality that few plants can tolerate (Maun 2009). Coastal primary sand-dune systems are phosphorus (P) deficient, high in sodium (Na), and organic matter (OM) is invariably low (Willis and Yemm 1961, Willis 1989). Gradients are often a key feature, salinity and pH decreasing away from the sea for example (Lane *et al.* 2008). Plant species

diversity is poor and density patchy, as is limited nutrient (Tilman 1984, Schaaf *et al*. 2011), in a structurally simplistic psammite matrix.

Arbuscular mycorrhizal (AM) fungi (mycobionts) are ubiquitous root symbionts of higher plants (phytobionts), associated with more than 90% of vascular plants and over 80% of all extant terrestrial plants (Wang and Qiu 2006). Primitive AM structures are described from 410 Ma Rhynie cherts in Aberdeen, Scotland (Kidston and Lang 1921, Remy *et al.* 1994) and the symbiosis is considered to have been indispensable in green plant colonization of land (Simon *et al.* 1993, Helgason and Fitter 2005). The association is the most widespread of extant terrestrial biomes plant symbioses. A primary functional attribute of the fungi in this intricate, delicate, dynamic and complex association is direct nutrient transfer *via* mycelium from soils to host plants, especially in hostile and nutrient depleted environments such as coastal dunes, and particularly where there is limited availability of low mobility resource P (Mosse, Hayman, and Arnold 1973, Bolan 1991, Smith *et al.* 1994), micronutrients (Clark and Zeto 2000), and ammonium- and nitrate-nitrogen (N) from organic matter (OM) (Hodge, Campbell, and Fitter 2001, Leigh, Hodge, and Fitter 2008). The function is in obligate acquisition of exchanged host-plant carbon (C).

Function is a cornerstone feature of ecological research activity (Huston 1997, Bengtsson 1998), how organisms and their bio-chemical reactions, and physical environments and their chemical reactions, respond to each other and interact through trophic levels bottom-up and top-down. Arbuscular mycorrhiza fungi are a keystone (see Glossary) phylum in soils (Read 2002, Jeffries *et al*. 2003), an influential element in the first trophic level that directly receives C from plant roots. The fungi constitute a large part of the standing soil C pool (Rillig *et al*. 2001, Zhu and Miller 2003) and are a ‘distribution centre’ of much of plant produced C throughout soil trophic levels *via* hyphosphere exudation. The phylum is a major

facilitator of soil nutrients through the symbiosis interface with higher plants. This thesis describes a research study in the efficiency of AM fungal nutrient-function. It is an assessment of spore abundance that may relate to how effective any of the AM taxa may be in nutrient delivery to host plants, undertaken in a primary dune system in Goa on the west coast of India.

Relatively few, compared to *in vitro* and pot culture experiments, AM fungal ecology field studies in dune systems are reported in the literature (e.g. Nicolson 1960, Fitter 1985, Johnson *et al*. 1991) despite a long history (Cowles 1899) of considerable research in plant community development (Connell and Slatyer 1977, Houle 1997). Those that are reported have generally described mycorrhizal status of selected plant species rhizosphere spore abundance, or root and soil colonization levels. Olsson and Wilhelmsson (2000) for example, reported rhizosphere soils AM fungal community structure from AM-signature phospholipid fatty-acid analysis. Kawahara and Ezawa (2013) employed a real-time polymerase chain reaction (PCR) technique to assess AM fungal community structure in individual plants. Few reports in the literature describe AM abundance in transect analysis in coastal dunes (e.g. Koske and Halvorson 1981, Rosendahl and Stukenbrock 2004). Research reported here may extend nutrient-function knowledge in what is believed to be the first enquiry of AM fungal sociobiology on a transect across a coastal primary sand-dune system on the Indian sub-continent.

Field-soil research studies are fraught with inherent difficulties. Even minimal soil and rhizosphere disturbance inevitably affects anthropogenic change at the micro-scale. Interpretation of single-factor role-play in the complexity surrounding the symbiosis is often confusing, and clear definition of experimental approach in isolating those factors tenuous. Below-ground functional biology is complex and there is probably a greater diversity of

biological organisms in soils than in any other sphere (Torsvik, Goksoyr, and Daae 1990, Dykhuizen 1998). Nevertheless the mycorrhizal ecologist must move into the field to investigate the role of AM fungi in the active ecosystem.

It was considered that the qualities of the experimental site may facilitate the study. Psammite soil is a clean medium that facilitates the labour intensive task of spore extraction. The annual AM life cycle is predictable where the monsoon growing season is short and swiftly followed by xeric conditions that encourage sporulation. Species richness in both AM fungi and host plants is generally low in nutrient-deficient primary dune systems (Grime 1979, Tilman 1982), fewer species associations perhaps displaying less complicated (although not necessarily less complex) community interactions. Almost all of the plant species are obligately mycorrhizic, and a visually obvious zonation in plant species across the dune system that is perhaps the outcome of edaphic gradients commonly associated with coastal sand dune systems (Kim and Yu 2009), may be mirrored by AM fungal diversity. Host plant species, edaphic factors and climate are drivers (see Glossary) of AM fungi (Johnson *et al*. 2005, Johnson *et al*. 2010, Gray *et al*. 2011). It is thus reasonable to assume that, in conducting an initial transect survey, and subsequent trials of rhizosphere soil nutrient-availability amendment, variance in spore diversity and abundance, increase or reduction, may equate with nutrient-function efficiency.

There is concern in using spore abundance as a criterion of AM fungal population density and the assumption that an empirical record of AM spores at genus and/or species level is analogous to nutrient-function may be suspect. The literature suggests there is no constancy in the relationship between sporulation and root distribution (Friese and Koske 1991, Olsson, Jakobson, and Wallander 2002), nor may there be between sporulation and soil mycelium density or functional activity (Zhao *et al*. 2001). Spore spatial and temporal distribution and

density may also vary in response to long- and short-term environmental variation (Gemma and Koske 1988, Escudero and Mendoza 2005, Ehinger, Koch, and Sanders 2009). St. John and Koske (1988) suggest AM fungal spores may occur in clumped distributions in the field, and thus the robustness of the study sampling strategy may be at risk. Furthermore, comparison between molecular and morphological survey has detected AM fungal species that may sporulate at a different time of the year from the time of sampling in field studies (Clapp *et al*. 2002). However, as Sieverding and Oehl (n/d) point out, "the spore number of individual AMF species certainly gives an indication on the fitness (see Glossary) of the species". Should any taxa be substantially greater in spore abundance in a nutrient-deficient dune system, it may suggest fitness is related to host-plant preference of association with fungal taxa that are most nutrient-function efficient.

1.2. Literature Review

1.2.1. Coastal sand-dune systems

There has been coastal sand grain deposition for as long as there has been river-sediment disgorge and tidal oceans in the geological history of Earth. Until plants invaded land it is unlikely there were existent dune systems as they are currently. It is plant roots and contiguous microflora that stabilize sand grains, forming a flexible natural barrier to encroachment by the sea. Coastal dunes geomorphological cycle is well documented (Pye 1983, Baas 2002). Prevailing onshore winds carry beach (strand) sun-dried surface sand grains to form a low incipient mound (berm) parallel to the water-line that highly adapted plant species colonize, accreting sand grains. The tenure of colonization is variable as on occasion the berm can be partially washed away (as sometimes happens in India west-coast monsoon season) by raised storm-surge sea-level or lunar high-tide. Gradually the embryo dune increases in height and breadth, wind pushing sand grains up the windward slope onto the crest that roll down the less-steep backslope, sheltering further and incrementally more diverse ecosystem development that can extend for tens of kilometers inland (Psuty 2008).

Primary coastal systems are hostile environments where nutrients are deficient and patchy, where high percolation rate and limited soil-water retention may affect plant-water balance, where high levels of low affinity uptake-mechanism Na may compete with potassium (K) uptake (Salisbury and Ross 1978) and upset osmotic balance, where aeolin sand grains can extinguish seedling development by burial (Maun 2009), and where intense solar heat and irradiance may adversely affect photosynthesis and C cycles (Powles 1984). Nevertheless adaptation of endemic plants enhances persistence in fecundity, which may indicate an element of stability (see Glossary) within the plant community, and hence overall function of the system.

Plant-ecology study of sand dune systems has a long history, pioneered by H.C. Cowles in his late 1890's work (Cowles 1899) on succession (seres) on the shores of Lake Michigan (later extended by Olson 1958). This has been followed by a considerable literature describing the complexities of lacustrine and coastal dune plant communities (e.g. Callaway and Walker 1997) and pedogenesis (e.g. Phillips *et al*. 1996). Generally the successional trends are from a limited diversity of patchy, prostrate herbaceous species at the near-shoreline through to inland forest over time, pH decreasing and OM increasing to a steady state in detectable gradients (Pennanen *et al*. 2001, Lane *et al*. 2008). The creepers *Ipomoea* and *Canavalia* dominate in tropical and sub-tropical foredunes, giving way to *Ammophila* spp., *Festuca* spp. and *Spartina* spp. amongst others in temperate regions over a latitudinal gradient (Hesp 2008). Stable secondary dunes establish further inland with increasing above- and below-ground community diversity and density. Many of the plant species in tropical and sub-tropical regions primary dunes are clonal in habit, often in combination with prolific seed production. The majority of grasses display C_4 photosynthetic metabolism (Collatz, Berry, and Clark 1998) and thus utilize limited soil moisture in high temperatures and irradiance, and low N conditions, more efficiently. Interestingly the literature suggests this evolutionary adaptation occurred only in relatively recent times, 25-32 Ma ago (Osborne and Beerling 2006), and where tropical primary dunes carry a high proportion of C_4 grass species in the extant community (Hesp 2008) ecosystem-function robustness may have been greatly enhanced by the event.

1.2.2. Arbuscular mycorrhizal fungi

Taxonomy of AM fungi currently describes 214 species in four orders, 13 families and 19 genera (Muthukumar *et al.* 2009), in the class Glomeromycetes of the phylum *Glomeromycota* (Schüßler, Schwarzott, and Walker 2001). Considering the phylum has remained extant for more than 450 Ma and is represented worldwide in all major terrestrial biomes (Treseder and Cross 2006, Rosendahl 2008, Rosendahl, McGee, and Morton 2009), this is a small number. Traditionally the species have been identified by morphological characteristics of spores and sporocarps, suspensors and adjacent hyphae and only since molecular methods have been used to elucidate phylogenetic relationships amongst the fungi has classification been clarified. The phylum was raised only in 2001 (Schüßler, Schwarzott, and Walker 2001), differentiated from similarly non-septate Zygomycotina and positioned relative to *Basidiomycetes* and *Ascomycetes* (Fig. 1.1). Transition in classification has occurred subsequently on a number of occasions (e.g. Redecker *et al.* 2013).

Almost all tropical plants are typically arbuscular mycorrhizal (Janos 1987) which may be relative to fast litter decomposition and consequent high ecosystem C turnover (Cornelissen *et al.* 2001). Few vascular plant families do not consistently host the symbiont, Chenopodiaceae, Polygonaceae, Juncaceae, Cruciferae (Brassicaceae), Caryophyllaceae and Proteaecae (Smith and Read 2008). The fungal species are considered non-host plant specific in their associations (Giovanetti and Hepper 1985) although there appear to be clear cases of preference (Vandenkoornhuyse *et al.* 2002, Sanders 2003, Kubota and Hyakumachi 2004, Croll *et al.* 2008). Preference, it is suggested here, may be relative to nutrient-function efficiency.

Fig. 1.1. Generalised taxonomic structure of AM fungi based on SSU rRNA gene sequences. A four-order structure for the phylum (*Glomeromycota*) is shown, with family ranking shown by ovals. From: Schüßler, Schwarzott, and Walker (2001).

Spores are thought to be multi-nucleate and heterokaryotic (Hijri and Sanders 2005), the fungi asexual (Pawlowska 2005, Croll and Sanders 2009). There is evidence that N (Motosugi and Terashima 2006) and water (Allen 2007) pass between hosts *via* a common hyphal network. Carbon that remains in the fungal structures within recipient host root is also translocated (Bago, Pfeffer, and Shachar-Hill 2000).

Intraradical hyphal modification characterizes two types of AM fungi, the Paris type (Plate 1.1f-i) that forms coils in host plant cortical cells, and the Arum type that forms arbuscules

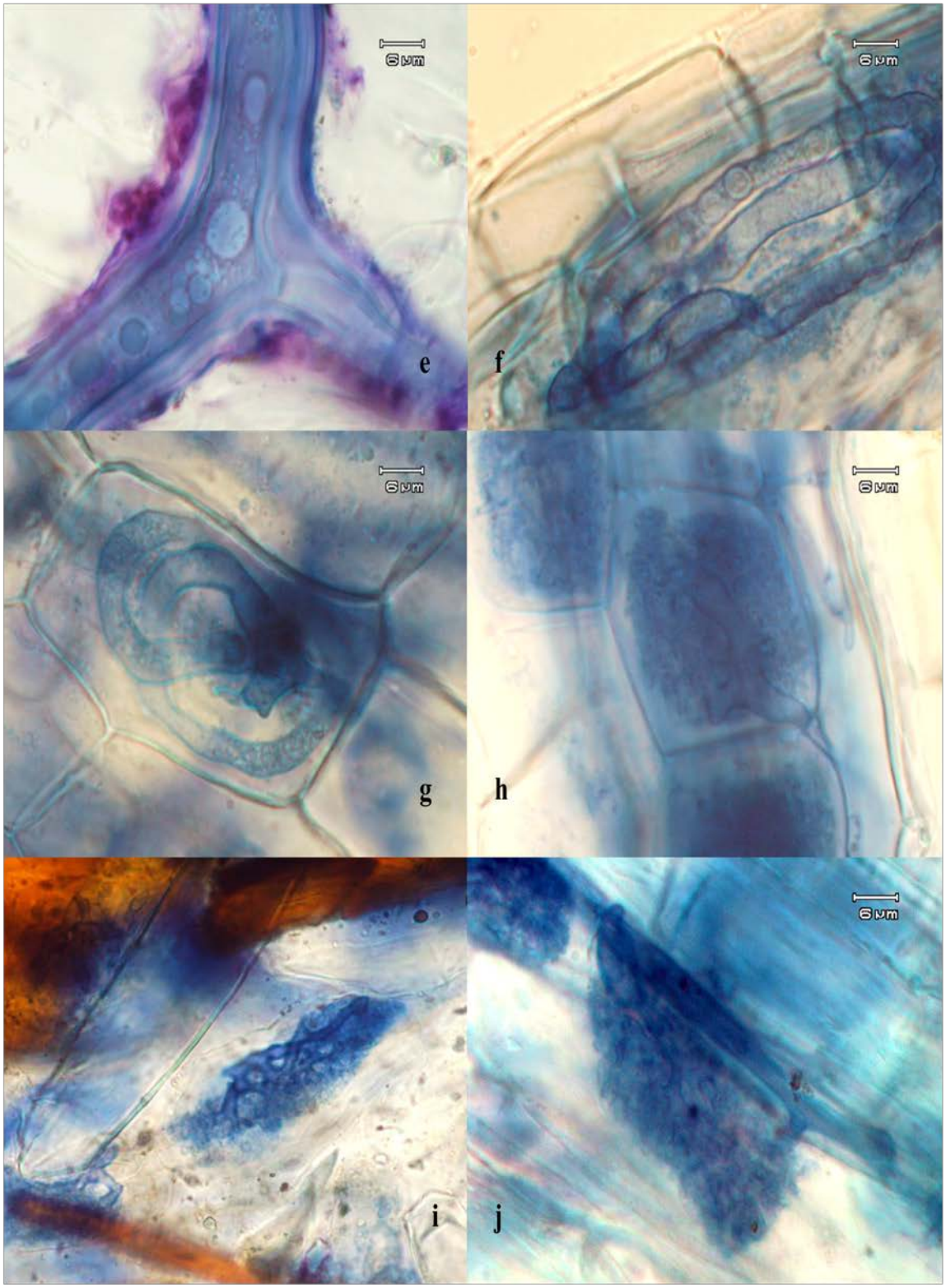


Plate 1.1. Intraradical structures of arbuscular mycorrhizal fungi. Legend: **e** Fluorescing lipid bodies in irregularly shaped thick-walled AM intraradical hypha. **f&g** AM *Paris*-type coils. **h&i** Intermediate *Paris*-type arbusculate coils. **j** *Arum*-type arbuscule.

(*frontis*, Plate 1.1j) that are characteristic tree-like structures from which AM fungi derive their name (Fig. 1.2). These are thought to be nutrient exchange sites, at least from fungus to host. Phosphate and ammonia/ammonium transporter systems, *via* an H^+-ATPase pathway, carry nutrients across the intracellular periarbuscular membrane formed with invaginated root

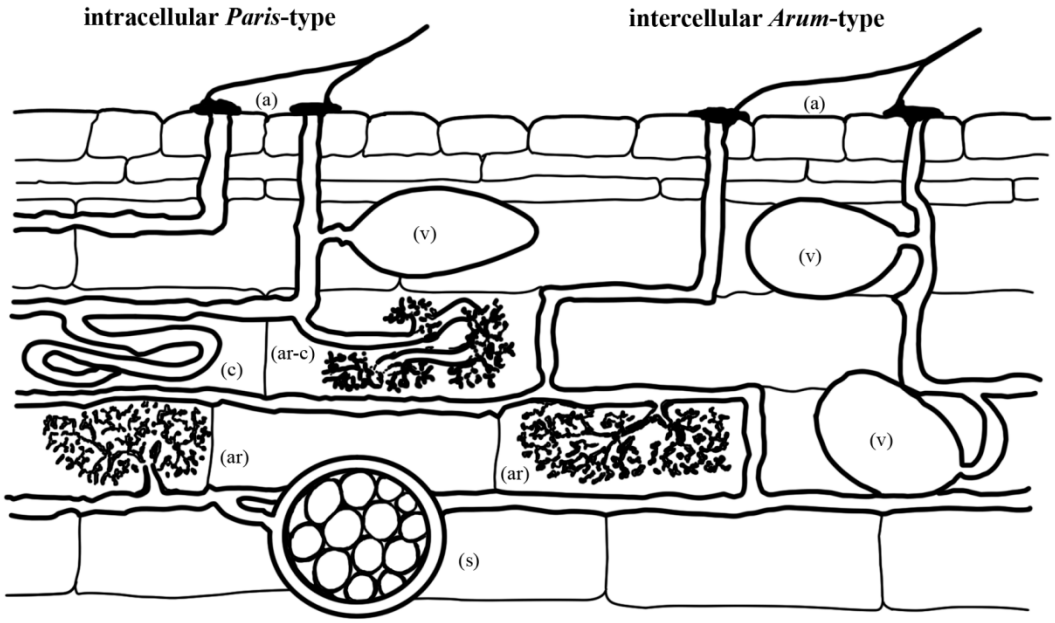


Fig. 1.2. Sketch of intraradical morphological features of AM fungi. (a) = appressoria; (ar) = arbuscules of intercellular *Arum*-type AM; (ar-c) = arbusculate-coil and (c) = coil of intracellular *Paris*-type AM; (s) = intraradical spore; (v) = vesicles.

cortex cell inner wall (Uehlein *et al.* 2007, Pumplin and Harrison 2009, Kobae and Hata 2010). These exchange sites are connected to a mycelial 'web' in the extraradical sphere by inter- and intra-cellular hyphae. All but Paraglomaceae, Archaeosporaceae and Gigasporaceae (which uniquely produce extraradical auxillary cells) species produce inter- and intra-cellular lipid-rich vesicles that possibly act as temporary storage organs, sometimes

converting to spore-like thick walled structures which may be of importance in the efficacy of root fragments as propagules (Plate 1.2a-d).

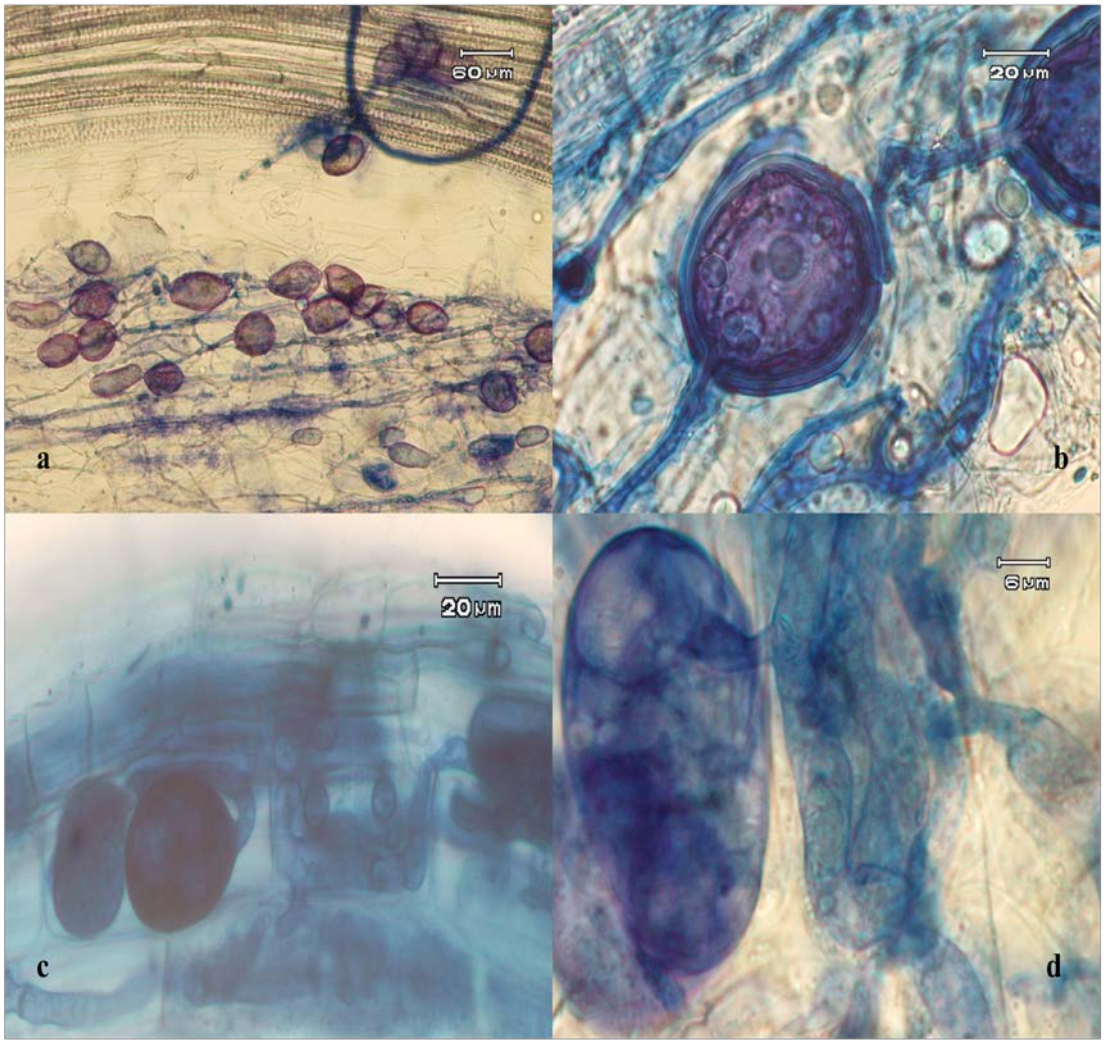


Plate 1.2. Intraradical structures of arbuscular mycorrhizal fungi. **Legend**: **a&b** Intraradical AM hyphae and vesicles. **c&d** AM vesicles adjacent to endophyte (DSE) knot and coil structures.

The extraradical mycelial network can be extensive, a peak measure of 111m cm^{-3} soil reported in tallgrass prairie by Miller, Reinhardt, and Jastrow (1995) and typically <1-26m g^{-1} of a variety of soils (Sylvia 1992). The species vary in the degree of soil volume occupied (Abbott and Robson 2006, Jakobsen, Abbott, and Robson 2006) and distance grown from the

host plant root (Munkvold *et al.* 2004). These may be pertinent features in the plants- and nutrients-patchy dune environment studied. Soil mycelial biomass does not necessarily equate to the quantities of nutrient transferred to hosts (Smith, Jakobsen, and Smith 2000). The life cycle (Fig. 1.3) is completed with the production of spores, a resting stage, tolerant of the extremes encountered in India west-coast dunes environmental conditions.

Arbuscular mycorrhizal fungal growth and development is dynamic and rapid. In the pre-symbiotic stage, which displays the lowest metabolic rate, a spore germ-tube may grow up to 20-30 mm. Root exudate triggers fan-shaped germ-tube branching after just a few hours (Tamasloukht *et al.* 2003) encouraging multiple entry points into a root. It may be that the spore is not the principal infective unit in thriving habitats however, root fragments and active hyphal networks being more effective (Smith and Read 2008). Appressoria are formed at points of contact with the root from which passive penetration of the epidermis occurs. Arbuscules, dichotomously highly-branched hyphae invaginating with the inner cell wall, may be formed within 1-6 days of penetration into cortex cells (Harley and Smith 1983) occupying when fully developed 35% and 36% of the cell in wheat and oats respectively (Alexander *et al.* 1988), dissolving, the host cell returning to its original form, after 4-15 days (Harley and Smith 1983). Kobae and Hata (2010) recorded only 2-3 days of active phosphate transport in transgenic rice roots before dissolution. Further arbuscules develop as intraradical hyphae spread through the root, branching and penetrating receptive cortical cells. This phenology suggests plant roots are soon colonized in the studied dunes when plant growth re-starts at the onset of monsoon.

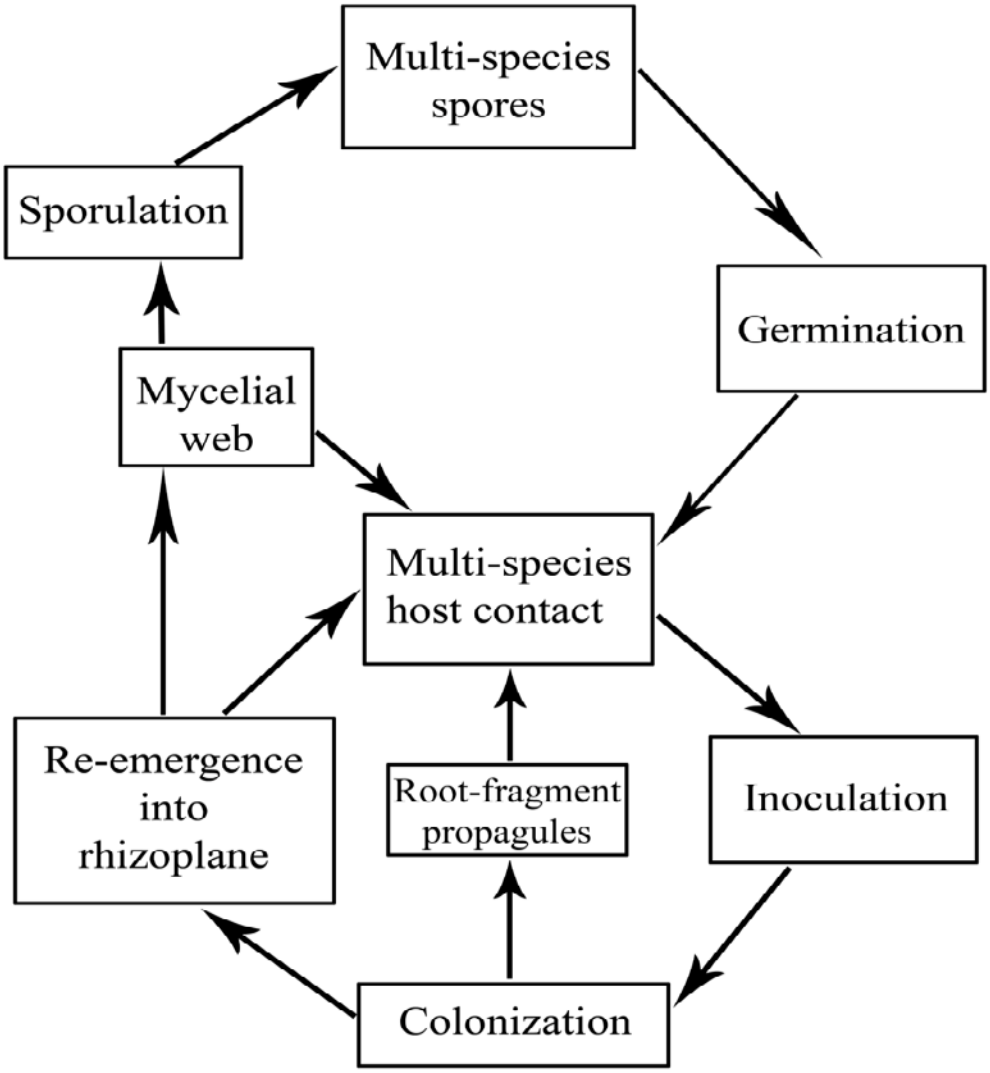


Fig. 1.3. General schematic of AM fungi life-cycle. The cycle varies temporally and spatially in all phases in response to variations in intra- and extra-radical environment.
Legend. In a thriving plant community AM fungal inoculation strategy includes colonization of roots by emerging extra-radical phase hyphal contact with roots of other plants in close proximity, by mycelium contact with roots further away, by root-fragment propagules i.e. intra-radical spores, vesicles and hyphae (**Plate 1.1b**), and by spore germination. After desiccation of host roots and mycelial web that occurs in west-coast India coastal dune systems, remaining viable spores are the majority of potential for inoculation of re-newed plant growth at the start of the next monsoon.

Total percentage of root occupied by arbuscules varies with effectiveness of the fungal species (Fitter 1985, Hart and Reader 2002), with season (Bohrer, Friese, and Amon 2004,

Hawkes *et al.* 2007, Garcia and Mendoza 2008), and with soil pH (Clark 1996, van Aarle, Olsson, and Söderström 2002), chemical properties (Posada *et al.* 2007), hydrology (Ray and Inouye 2006, Schreiner, Tarara, and Smithyman 2007) and temperature (Smith and Read 2008, Bunn, Lekberg, and Zabinski 2009) statuses, with soil biota interactions (Dauber *et al.* 2008, Schreiner and Pinkerton 2008) and with host plant species (Toth *et al.* 1990, Klironomos 2003, Nijjer *et al.* 2008), host phenological stages (Pongrac *et al.* 2007) and C allocation (Allsopp 1998, Staddon and Fitter 1998, Muthukumar and Udaiyan 2000). AM nutrient function might be driven by any single, or any combination, of these factors.

The extraradical mycelia branch and extend into the rhizosphere and beyond when arbuscules have been formed (Smith and Read 2008) which implies photoassimilates cross the periarbuscular membrane. Runner hyphae extend alongside and around the root apically and distally, re-inoculating. Feeder hyphae extend out into the matrix bridging rhizosphere nutrient depletion zones, branching dichotomously up to eight times, reducing in diameter from *ca* 20 to 2 µm (Friese and Allen 1991) enabling penetration of soil crumb far beyond that of roots, hugely increasing soil nutrient absorption volume over root hairs. The extensive mycelial web formed may directly or through anastomosis of compatible species connect the root systems of plants of different species, genera and families (Giovannetti *et al.* 2006) which may have considerable implication upon plant community factors in all habitats. Several species of a number of genera may inoculate a host plant simultaneously (Bever *et al.* 2001), all of which are competitive with each other (Wilson 1984, Smith and Read 2008) yet can be complimentary (Jansa, Smith, and Smith 2008). Each species displays difference in colonization strategy at the taxonomic level (Hart and Reader 2002) and may possibly contribute differing functions to the symbiosis to varying degrees.

Species of AM fungi have been implicated in enhancing plant Na tolerance in saline coastal-dune soils, a review by Evelin, Kapoor, and Giri (2009) describing several mechanisms employed including enhanced mineral nutrient uptake, maintenance of K : Na ratio (see reference Salisbury and Ross 1978 above), and biochemical, physiological and genetic changes in host plants. Hammer *et al.* (2011) found *Glomus intraradices* N.C. Schenck & G.M. Sm. extraradical hyphae selectively excluded uptake of toxic Na *in vitro*. Such a function would be highly beneficial to plant fitness in coastal dune systems where Na deposition can be constant and often in high concentration.

Unusual in symbioses, control in nutrient exchange can be undertaken by either plant or fungus (Smith and Smith 2012). Transport of phospholipid materials within hyphae is bi-directional (Smith and Gianinazzi-Pearson 1988), soil derived nutrients to hosts, carbon assimilates from hosts utilized by the fungus and distributed throughout the mycelial web and hyphosphere. Where the fungal partner is able to deliver excess nutrient, host physiological changes have been observed, e.g. significant increase in transpiration and photosynthetic rates (Allen *et al.* 1981). The host then, it was suggested, is able to similarly return excess C.

Evidential concensus indicates that all species of AM fungi contribute, to a greater or lesser degree, with each plant species variably responding to the fungal symbiont colonizing species (Smith *et al.* 2010), inorganic P (P_i) to their hosts. This is particularly observed in a P limited environment such as coastal dunes. There is evidence of mineralisation of organic P (P_o) by AM extraradical hyphae (Koide and Kabir 2000) but Joner, Van Aarle, and Vosatka (2000) found there were very limited quantities of AM fungal derived phosphatase in soils adjacent to hyphae. Other soil microorganisms, bacterial and fungal, with abundant P_o conversion capacity, mobilize P_i into the labile pool that AM fungi trans-membrane transport into extraradical hyphae (Karandashov and Bucher 2005), convert to polyphosphate long-chain

and granular fractions (Solaiman *et al.* 1999) and transport to the intraradical exchange sites by cytoplasmic streaming (Cox *et al.* 1980).

Carbon from the host is derived from plant sugars. The mode of transport to the symbiont is thought to be passive efflux, intraradical organs taking up and using hexose, a substantial amount of which is used in lipid synthesis (Bago, Pfeffer, and Shachar-Hill 2000). Up to 20% of total photosynthate might be exchanged (which is an indication of the functional importance of the symbiosis to mycotrophic plants), always of recent assimilate. Flux of lipid bodies (Plate 1.1e) from the intraradical exchange sites within the cortical cells to extraradical hyphae has been imaged by real-time immunofluorescence technique (Bago *et al.* 2002a) and movement in both directions shown in other work (Bago *et al.* 2002b). Much of this C is utilized in mycelium maintenance and growth, and spore development, and there is evidence that just as roots exude or leak C into the rhizosphere soil matrix influencing biotic populations (Jones, Nguyen, and Finlay 2009), the AM mycelial web releases C into the mycorrhizosphere (Toljander *et al.* 2007). There is evidence to suggest, for example, that phosphate solubilizing bacteria (PSB) taxa and populations may be influenced by AM fungi (Andrade *et al.* 1997), and N_2-fixing *Rhizobium* species (Barea *et al.* 2005). Toljander *et al.* (2007) not only identified carbohydrates exuded from AM mycelium but clearly demonstrated direct effects upon soil microbial community.

Comparatively recent investigation into N transport from OM and leaf litter (Aristizábal, Rivera, and Janos 2004, Leigh, Hodge, and Fitter 2008) to hosts has shown AM to be a major component of the N cycle. Unlike in the P relationship, AM fungi do not acquire more N at low soil-nutrient levels (Reynolds *et al.* 2005) but can make a significant contribution to plant N requirement (Hodge and Fitter 2010), particularly in dry soils such as the study site between monsoons where mobility in the labile pool to the direct pathway through roots is

restricted (Tobar, Azcón, and Barea 1994). The hyphal pathway converts inorganic N taken up from the labile pool into amino acids, translocated principally as arginine from extraradical to intraradical hyphae (Govindarajulu *et al.* 2005) where N is converted to ammonia before passing to the host. A recent report by Guether *et al*. (2011) describes the characterization of an organic N transporter in *Lotus japonicus* L. (Fabaceae) roots induced by mycorrhization that may be involved in active transfer of organic N compounds, principally energy rich amino acids, to the plant.

Micronutrients are also directly transported to host plants. Suzuki *et al.* (2001) detected, using a multitracer technique, the uptake of Na, zinc (Zn), selenium (Se), rubidium (Rb), and strontium (Sr) in AM hyphae. Bürkert and Robson (1994) described variable Zn uptake in three fungal species. Caris *et al.* (1998) reported uptake of iron (Fe) in sorghum by *Glomus mosseae* Gerde & Trappe but not in peanut, and Marschner and Dell (1994) up to 60% of plant copper (Cu) and 10% K requirements in experimental chambers. Clark and Zeto (2000) found K, calcium (Ca) and magnesium (Mg) uptake enhanced in acidic soils and Li, Marschner, and George (2006) found an increase in plant shoot Cu in calcareous soil, relative to P uptake. Allen and Shachar-Hill (2009) found, in monoxenic culture of *Daucus carota* L. transgenic roots with *G. intraradices,* uptake of sulphur (S) halved when supply of the nutrient was increased to above growth-limiting levels.

There is considerable evidence that the Glomalean hydrophobic glycoprotein glomalin is intrinsically involved in soil aggregation (Rillig and Mummey 2006) which may have far-reaching implications in psammite soils. Secretion from active extraradical hyphae and deterioration of moribund hyphae contribute large quantities of this recalcitrant hyphal wall material into the soil matrix where it acts as a mucilaginous glue. Micro-aggregates are bound tightly, looser macro-aggregates (>250 μm) temporarily more loosely bound as small

particles (Smith and Read 2008). The prior colonization by myriad soil microbiota and subsequent incorporation of detrital organic materials develops and maintains a structurally water-stable living soil, a sequence which might well be analogous to the era of terrestrial plant invasion, and to the development of dune-system seres.

1.2.3. Functionality and AM fungal research

Functional ecology (Bradshaw 1987, Calow 1987, Grime 1987) is the study of how organism traits relate to each other and to their environment, on temporal and spatial scales from global to micro-niche level. This study follows the pragmatic three-part description of the discipline proposed by Keddy (1992): a) constructing trait matrices through screening; b) exploring empirical relationships among these traits; and c) determining the relationships between traits and environment. The science is an integrative principle of disparate disciplines, evolutionary biology, molecular ecology, metapopulation theory and traditional ecological studies, and development of the ecosystem concept has shifted ecological research beyond pattern description towards a greater understanding of how diverse genotypes interact.

Arbuscular mycorrhizal fungi are the most widespread of all known plant symbioses. The phylum's functional roles, besides that of nutrient facilitation explored in this study, include reduction of root invasion by microbial soil-borne plant pathogens (Newsham, Fitter, and Watkinson 1995), reduction in plant uptake of phytotoxic heavy metals (Göhre and Paszkowski 2006) and trace metals including Zn (Christie, Li, and Chen 2004), improved host plant water balance in periods of ample water and drought (Augé 2001) and, as described above, soil particle aggregation through the cohesive action of a Glomalean water-stable glycoprotein (Rillig and Mummey 2006). Further effects of AM fungal association reported include reduction in insect herbivory by induced plant response (Bennett, Bever, and Bowers 2009) and variation in that response relative to N uptake (Gange, Brown, and Aplin 2005), increase in insect pollination (Gange and Smith 2005) and percentage increase in F_1 generation seed germination (Srivastava and Mukerji 1995). AM fungi are also reported to increase the density of insect herbivore parasites in trophic food webs (Hempel *et al*. 2009, Hoffmann, Vierheilig, and Schausberger 2010). There is evidence to suggest AM fungi may

play a significant role in soil N and C cycles (Govindarajulu *et al.* 2005, Jones, Nguyen, and Finlay, 2009) and make considerable contribution to terrestrial ecosystem C sinks (Wright and Upadhyaya 1998). In addition to the above functions, AM fungi can also influence, perhaps even organize and structure, plant community patterns (van der Heijden, Bardgett, and van Straalen 2008) and soil microbiota community populations (Rillig *et al*. 2006, Toljander *et al*. 2007). Such evidence indicates Glomeromycotan functional relationship with plants and soils is an integral and essential factor in overall ecosystem development and maintenance.

The phylum may not necessarily, however, be the principal or only driving force in the system. Studies have indicated that AM may have been driven by plants (Johnson *et al*. 2005), particularly in the case of invasive plant establishment (Hawkes *et al*. 2006, Burke 2008), that AM were driven by soil edaphic factors (Cavagnaro *et al*. 2005), some where AM have influenced plant community (Francis and Read 1995, Van der Heijden *et al*. 1998, O'Connor, Smith, and Smith 2002) and others where AM influenced soils (Wright and Upadhaya 1998, Van der Heijden, Wiemken, and Sanders 2003). Nor is it to say the AM fungus is a benign mutualist. There have been reports of non-benefit C-cost to hosts (Bever 2002, Paszkowski 2006) and Jones and Smith (2004) concluded that mycorrhizas may occupy positions along a parasitism – mutualism continuum. Willis, Rodrigues and Harris (2013) further suggested temporal variation on such a continuum as the symbionts respond to fluctuating environmental influence. The quest for C is as much a part of fungal strategy as that for any other nutrient.

Nevertheless, the range of facilitative functions outlined above, although mostly assessed in controlled experiments and so removed from the complexity of trophic levels, co-evolvement with plant roots and the phylum's influence on soil biotic and abiotic factors affords a

meaningful research opportunity in the functional ecology of AM fungi. This study's 'next-progressive' step is an attempt to extend knowledge of the phylum's nutrient-function role by assessing the efficiency of individual AM fungal taxa in a hostile dune-system environment.

1.2.4. AM fungi in primary coastal sand-dune systems

The AM fungal nutrient functions described above confer considerable contribution to plant fitness in the hostile, nutrient deficient environment of primary dune systems. Although none of the plant species may be obligately mycorrhizic, it is likely that few primary dune system plants would thrive without AM association (Koske and Polson 1984, Koske *et al*. 2008) and seed fecundity and vegetative propagation would be severely limited. Chapin (1987) suggested that even plants adapted to low-nutrient habitats have a low capacity to acquire less mobile phosphate and ammonium ions, nutrients that AM fungi are particularly adept in scavenging and transporting to hosts. The fungi are therefore essential to the nutritional welfare of individual plants and to the maintenance of overall system stability.

The most prevalent AM fungal genera in primary coastal dunes worldwide are *Acaulospora, Gigaspora*, *Glomus* and *Scutellospora* (Maun 2009). Reported spore densities per unit volume of soil are variable depending on season, host genotype and phenology, and environment, ranging from 1 to >300 100 g^{-1} soil (Maun 2009). The greater the number, the higher is the mycorrhizal inoculum potential (MIP) (see Glossary) in the soil, and the sooner germinating seeds or vegetative propagules may come into contact with spores, enhancing earlier plant growth benefit. This may be particularly important in a patchy tropical-monsoon primary sand dunes environment. In effect AM extraradical hyphae bridge the spaces between root and patchily distributed nutrient.

Arbuscular mycorrhiza community structure is seldom investigated relative to plant community structure and spatial and temporal soil chemistry at fine scale (Wiens 1989), and it is considered such a research program in a nutrient deficient, simple psammite soil, and climatically predictable tropical monsoon coastal dune system may extend knowledge of AM function. Dunes studies (Maun 2009) have generally shown that low concentrations of OM

and P in particular limit plant growth, and AM fungi are known to effectively facilitate plant NH_4 and PO_4 uptake in deficient soils (Smith and Read 2008). The data may also reveal detail of seasonal edaphic and spore variation, and novel insight into the structure of the ecosystem, into AM community structure, and into the tri-partite relationships of plants, soil chemistry and AM fungi. From analysis of data it may be possible to examine nutrient function in AM at the taxonomic level, and whether, if any, AM taxon is (are) particularly efficient in supplying sand-dune deficient nutrients to host plants.

The hypothesis is that certain AM fungal taxa are more efficient at providing soil nutrients to host plants in this scarce-resource primary dune system, and that the most abundant spores recovered may represent those taxa.

1.3. Objectives

1. To conduct an interrupted belt-transect survey of plant species, soil nutrient concentrations, and AM spore abundance and diversity.
2. To conduct experiments testing the effect of nutrient amendment to pot-contained rhizospere soils of selected plant species, in the field, on spore abundance and diversity.
3. To analyse plant community structure.
4. To examine variation in soil nutrient concentrations over space and time.
5. To record and statistically examine abundance and diversity of AM fungal spores.
6. To assess relationships between plants, edaphic factors, and AM.
7. To explore indication of AM nutrient-function efficiency.

Chapter 3 describes a survey of an interrupted belt transect marked across the width of the dune system along an observed plant species zonation, substanially achieving Objective 1, contributing to Objectives 3 and 5, and providing data used in Objective 6 and the more speculative Objective 7. Chapter 4 describes a preliminary experiment initiating Objective 2, a study of the effect on AM fungal spore abundance of three substrate nutrient amendments in pots placed beneath ramets of two perennial foredune species further contributing to Objective 5, examination of spore and plant variance from control further contributing to Objective 6. Chapter 5 reports a succeeding experiment reinforcing Objective 2, in this instant one amendment only introduced into the rhizosphere soil of a single plant species along the Chapter 3 transect, spore abundance and diversity variance against control pots again contributing to Objective 5 and 6. Comparison of data from transect survey and the two experiments contributes substantially to Objective 6, and the speculative Objective 7.

CHAPTER 2

GENERAL MATERIALS AND METHODS

Goa, a Portuguese enclave for 450 years until 1972, in terms of area the smallest State of India, lies on the west coast of the sub-continent between longitudes 74º 20' 13" and 73º 40' 33" E and latitudes 15º 48' 00" and 14º 53' 54" N, covers 3702 km², has a 105 km coastline and is 65 km at its widest. It is bounded on the west by the Arabian Sea, on the east by the Western Ghats. It is bordered to the north by the state of Maharashtra and to the east and south by Karnataka. Topographical range is from sea level to 1166 m at the highest point (Sonsagar), average elevation 800 m. Geologically Goa is complex and varied. The basement rock is early Archeaen, tonalitic trondhjemite gneiss (Plate 2.1) and greenstones with younger

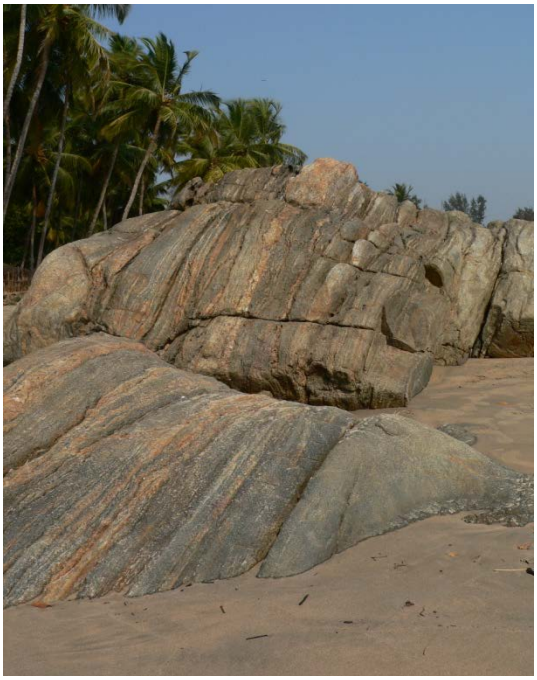


Plate 2.1. Basement trondhjemite gneiss exposed on Patnem beach, S. Goa.

intruded greywackes and granites (Dhoundial *et al.* 1987, Fernandes 2009) that have been extensively laterized, particularly from the higher plateaus down to the sea. Physical

geography divides Goa into four distinct regions, *ca* 600 km² of mountainous terrain to the east running more or less parallel to the coast, the central plateaus at 100-30 m elevation, the low lying river basins and coastal plains, and the beaches and dune systems. The two largest rivers, Zuari and Mandovi, with their tributaries, drain 69% of the State and are navigable to shipping throughout the year. Five other major rivers are Tiracol, which marks the border with Maharashtra, and Chapora in the north, Sal, Talpona and Galgibaga in the south. All seven sources are in the Western Ghats. The longest, River Mandovi, is only 77 km and the total navigable length is 253 km. The dominant climatic feature is the India west coast monsoon that begins in early June (Varkey 2007). Annual mean precipitation on the coast is 2770 mm, 2500 mm of which falls before the end of September. At the foothills of the Western Ghats the annual mean is >4000 mm, again *ca* 90% falling June-September. Temperatures range from winter (December/January) minimum ~12ºC at night to April/May minimum ~27ºC, daytime temperatures averaging ~30ºC+. Relative humidity (RH) increases from ~50% in winter to ~72% in early April. From mid-April to the onset of the heavy and continuous monsoon rains in early/mid-June, summer, occasional light showers can occur, more regularly, and intense, as June approaches. Monsoon inception instantly reduces temperatures by ~10º whilst RH nears 100%. Heavy and continuous rains continue into August, slowly changing to less regular, often still heavy, showers. Rainfall after November is rare.

The research was undertaken between 2010 and 2012. The field-work in the dune system was carried out during consecutive monsoons from the beginning of June to November/December each year, and subsequent analyses of field collections, stored at 4ºC until examined, in the following months. Auxiliary pot-culture experiments were conducted

throughout each year at the Goa University, both in a purpose-built culture house and in the University grounds.

The site selected for field work is on an aggrading coastline (Mascarenhas 1998) at Morjim in north Goa, India (Grid Ref. 15º37´55" N, 73º43´22" E) in a bay that is approximately 1 km long and 2 km north of the largest fresh-water outlet in north Goa, River Chapora (Fig. 2.1).

Fig. 2.1. Geological map of Goa (Fernandes 2009) showing the study-site location.

The river drains phyllite in its upper reaches and quartzite in the lower and the continental shelf P_2O_5 content ranges from 0.03% to 1.19% in the inner shelf sediments, showing positive correlation with Al_2O_3 and Fe_2O_3 (Rao and Wagle 1997). The perpendicular to the shoreline interrupted belt transect selected (Fig. 2.2) lies *ca* 80 m from the southern end of the bay, is until now little affected by a booming tourist industry, and extends *ca* 175 m from the

shoreline, after which is cultivated rice-paddy. The line begins at a single *ca* 4-7 m high, 35-45 m wide grey dunes ridge with a narrow, annually-formed incipient yellow dune in front that is partially or, in heavy monsoon seasons, wholly washed away by strong tides that

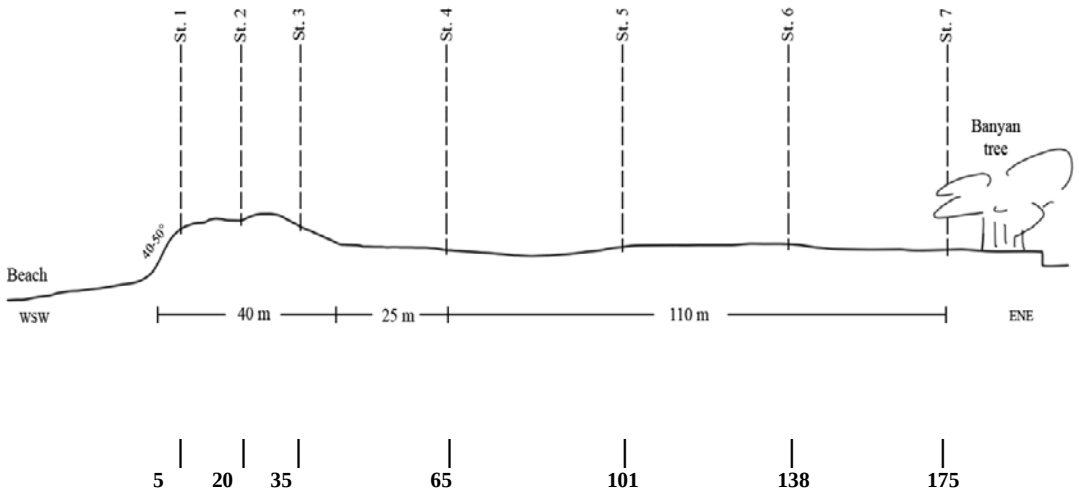


Fig. 2.2. Transect line on Morjim (Goa) dune system indicating positions of sample sites (St. 1-7) in metres from mean high-water mark (MH-WM).

also cut scarps (see Glossary) into the dune face. Behind is a 130-140 m level area that undulates by 20-50 cm on a gross scale, by a mm fine-scale on the surface, sloping down gently before the cultivated land. There are no secondary dunes on the line of the transect but there are towards the mid-bay region behind which are village dwellings. There is no development of slacks, the water-table rising to the surface for a brief period in monsoon of 2011 only, and that not on the line of the transect. The system is sparsely planted with coconut (*Cocos nucifera* L.) and patches of cashew (*Anacardium occidentale* L.), and is sporadically grazed by domestic animals. The line excluded all trees and shrubs and the stations followed what appeared to be a distinct zonation of plant species. Most of the plant species endemic to the system display physiological or anatomical features of hostile-environment tolerance, annuals are ephemeral, all transect-encountered plants are prostrate,

and patchy in distribution of limited species diversity. The stations were not rigidly lined out as inserted pegs had previously been removed but care was taken to ensure all sampling was undertaken within a *ca* 2.5 m width and 3 m transect length from unobtrusive markers at each station. Plant rooted frequency (see Glossary), the recorded "*local frequency*" presence or absence of individuals (Greig-Smith 1983) found rooted within each of the inner 100 mm x 100 mm squares of a 600 mm x 600 mm grid-quadrat was used (hereafter referred to as 'plant frequency'), rather than a cover (see Glossary) procedure that may have amplified sample error (Greig-Smith 1983). Phyllotaxy is highly variable, even amongst the grass species, and may have been a less representative measure. Means are expressed as percentage frequency from five samples in each station (i.e. 5 x 12 100 mm squares), the quadrat tossed (rather than placed which may have raised sub-concious pre-disposition bias) in such a manner as to be representative, within sample error parameters, of the whole of each station area.

In each station rhizosphere soil samples were extracted, five samples from each of the dominant plant species, after scaping away surface layer, to a 20 cm depth and taken from all quarters of the plants. The accrued sub-samples were thoroughly mixed by hand to give a representative heterogeneous mean sample from each station whilst minimizing sample error. It was considered gradients in edaphic factors might be discerned along the transect line. It was anticipated there might be several: pH, salinity, ammonium-N (NH_4-N), perhaps OC. Air-dried soil sub-samples were sieved to 2 mm to remove large OM fraction, a common procedure that, it is suggested, results in a higher proportional determination of recalcitrant (see Glossary) material (Six *et al*. 2002). The samples were submitted to Government of Goa Agricultural Department, Soils Analysis Laboratory, Ela Farm, Old Goa for chemical analysis. Analyses followed procedures laid out by Singh, Chhonkar, and Dwivedi (2005) for pH in soil-water suspension 1:2 ratio (Elico L1 120 pH meter), EC from the clear extract after

pH measurement (Elico CM 180 conductivity meter), OC by the Walkley and Black (1934) method, factored to give OM percentage by weight, available P by Brays Method (1945) using Bray's No. 1 solution, potassium (K_2O) and Na by the ammonium acetate method from Hanway and Heidel (1952), and Mg and Ca by a modified ammonium acetate method from Barvah and Barthakur (1997) (Elico flame photometer Unit 21). Available micro-nutrients Zn, Cu, manganese (Mn) and Fe were assessed by the DTPA-$CaCl_2$-TEA method (Lindsay and Norvell 1978) using an atomic absorption spectrophotometer AAS-EC Electronics Corps of India Element AS AAS 4139. Total-N, NH_4-N (LOD 0.0006% - Loan *et al*. 2013) and nitrate-N (NO_3-N) partial-transect data Stations 4-7 were gained by titration-method procedures described in Indian Bureau of Mines (2004) and analysed by Italab Ltd., Madgaon, Goa. The samples of each station submitted were 100 g of 2 mm-sieved material that removed larger OM material, from which further sub-samples were extracted by the agencies. Limits of detection (LOD) were not available. Samples were not submitted for determination a second time.

The field sampling method for the extraction of spores was the same as for soil chemical analysis, with an additional extraction of three 50 g sub-samples from each homogenized field sample that were stored at 4ºC until processed. Just as with soil analysis samples, the sampling method was devised to reduce sampling error as much as possible but without loss of representative heterogeneity. Rhizosphere soils have been shown to contain a higher density of AM spores than surrounding soils in patchy plant communities (Sylvia 1986) and by sampling the four quarters of the plant rhizosphere all of the taxa present should be encountered. There are, however, issues with the use of spore density being representative of AM fungal population (Kowalchuk, De Souza, and Van Veen 2002). As pointed out above the relative abundance and ratio of spores to mycelial web can be highly variable between

taxa that might increase sample error, as might the ‘clumping’ sporulation pattern referred to (Friese and Koske 1991). Spore retrieval from sub-samples followed a modified Gerdeman and Nicolson (1963) procedure where 355 and 37µm sieves only were used. It was considered there may be some AM spores and/or sporocarps i.e. those above 355 µm in diameter, lost but earlier trials using a full range of nested sieves (730, 550, 370, 250, 150, 106, 75 and 37µm) elicited no loss at all and the gain in time was considerable. Sub-samples (50 g) from fresh or fridge-stored (4ºC) field soil samples were placed in a container and tap water added, stirred gently but thoroughly using a glass rod, sediment displaced for 15 seconds only and the aliquot gently poured through the sieves. The procedure was repeated a minimum of seven times for each sub-sample. The residues collected in the 37 µm sieve were then carefully washed into a beaker. The resulting aliquot was funnel-filtered through Whatman No.1 filter paper and clean, fresh, whole (i.e. little or no detrital attachment, strong atypical colour, no damage or fracture and contents confirmed present by tweeezer-pressure) spores were retrieved by picking-out with fine tweezers (Du Mont, Switzerland) by a modified Gaur and Adholeya (1994) intersect method that ensured inspection of the total residue under a binocular microscope at 45x magnification. Species identities were verified by examination of specimens’ morphological characteristics, whole and very carefully broken spores, mounted in water, polyvinyl alcohol-lactic acid-glycerol (PVLG) and Melzer’s reagent in PVLG (Koske and Tessier 1983) under cover-slips, using a compound microscope and micrographs from an attached digital camera (see below for specifications).

Initially identification was carried out to a traditional (prior to phylum re-classification in 2001 [Schüßler, Schwarzott, and Walker 2001]) general method of spore classification at ‘genus’ level where the genera *Acaulospora* and *Glomus* stand alone, and *Gigaspora* and *Scutellospora*, the two genera comprising the family Gigasporaceae, are combined and

referred to as the “Gigasporaceae genus”. This classification is used throughout the thesis. Each category is readily recognized, *Acaulospora* species by remnant saccule unique to the genus, surface ornamentation, and no suspensor or hyphal attachment (Plate 2.2a,b), Gigasporaceae by the bulbous suspensor unique to the family (Plate 2.2c) (a germination shield found only in *Scutellospora* [Plate 2.2d]), and *Glomus* species by a ubiquitous and conspicuous sub-tending hyphal attachment (Plate 2.2e,f). The classification concurs with the worldwide prevalence in coastal dunes referred to above (Maun 2009).

Identification at species level requires considerably more time. Here further morphological characteristics need scrutiny, for example colour, size, number and thickness of walls, pattern of ornamentation in *Acaulospora*, shape of suspensor in *Glomus* and position and shape of the septum formed to retain spore contents when fully developed, shape and pattern of germination shield in *Scutellospora* spp., reaction to Melzer’s reagent, size and quantity of phospholipid globules ejected from a broken spore. Species are separable by morphological chararcteristics but there have been problems in closely related species that could only be separated by molecular methods that have prompted much recent re-assessment of the phylums phylogeny, and continues to do so. Reference was made to Morton and Benny (1990), Rodrigues and Muthukumar (2009), Schenck and Pérez (1990), International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (n/d) and the commendable Neue Seite 1 (n/d).

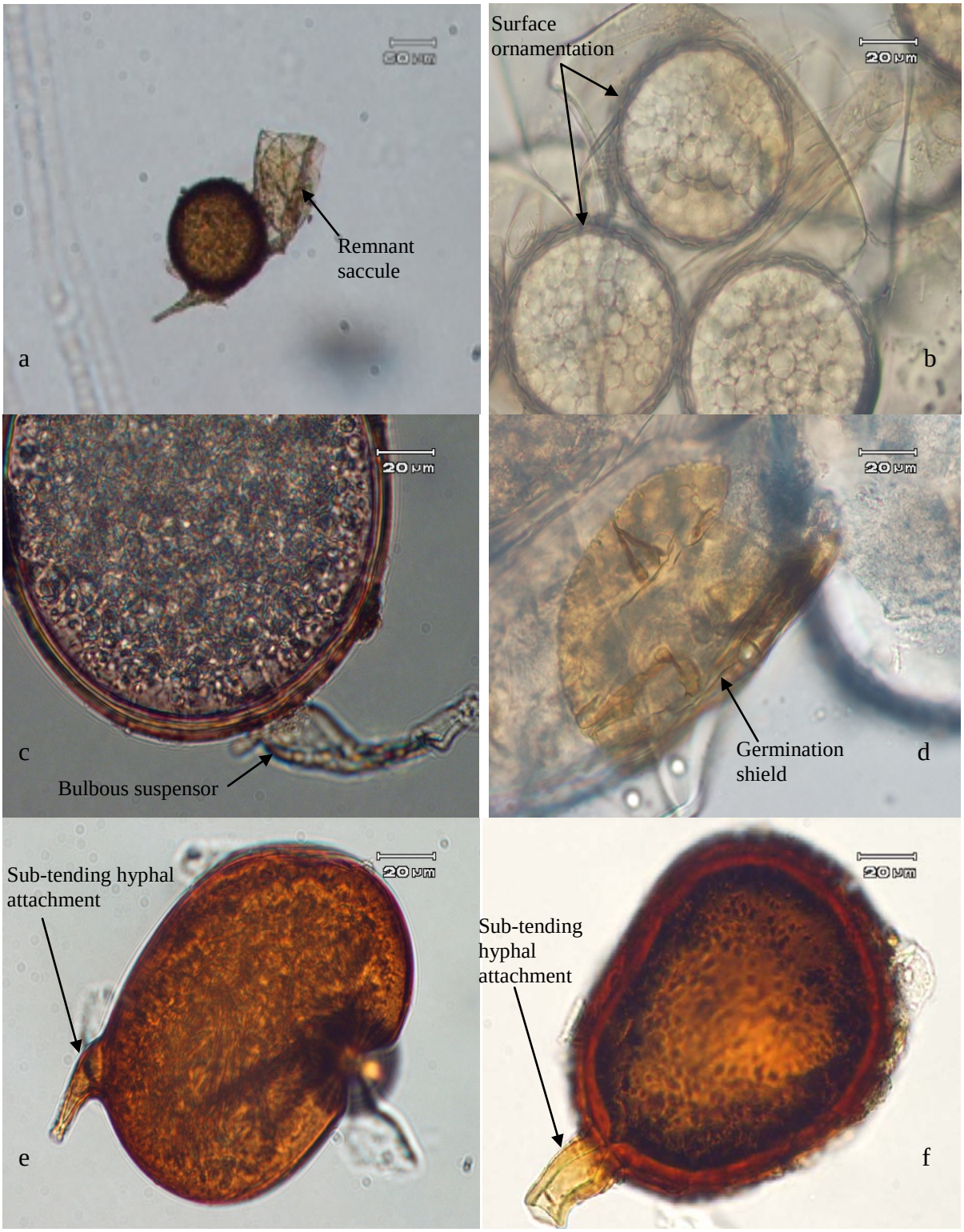


Plate 2.2. Micrographs of AM spores that represent the 2 genera and 1 family taxonomic "genus" categorization applied in the text. **a&b**) *Acaulospora* spp. **c**) *Gigaspora* sp. and **d**) *Scutellospora* sp., the 2 genera that comprise the family Gigasporaceae **e&f**) *Glomus* spp.

Root colonization was assessed by observation of presence or absence of AM non-septate hyphae, arbuscules, coils or vesicles by a trypan-blue staining technique from Phillips and Hayman (1970). Representative field- and pot-grown root material samples, fine-root sub-samples removed with scissors disturbing the subject as little as possible if examining interim colonization in field- or pot-plant subjects, or the fine roots of whole plant systems if uprooted, were gently washed in water to remove soil and organic particles before cutting into 1 cm pieces, clearing in 2% KOH at 90ºC for 45-60 min, thoroughly rinsed in water, fixed in 5N HCl for 10 min and left overnight in trypan-blue stain (0.05 g trypan-blue; 50.0 ml lactic acid; 10 ml glycerine; 40 ml DH_2O). Sub-samples were laid onto glass slides in PVLG with cover slips, gently pressed, and viewed through the compound microscope. Trypan-blue binds to plasma proteins in the fungal structures which are stained light- to dark-blue. Colour intensity can vary among species. It was found that different plant species require varying clearing time, too little and background root cell tissue will also stain blue obscuring the objective, too much and the fungal and root tissue disintegrates. The procedure of enumeration of colonization level was carefully considered. There are several methods commonly used for quantifying AM fungal colonization of root tissue, for example the slide method (Giovannetti and Mosse 1980) and the line-intersect method (McGonigle *et al*. 1990), all of which, it was judged, result in estimates of AM fungal colonization that differ and thus are not comparable. The discrepancy is due not only to the techniques of quantification but the dynamism of the symbiosis. To report figures on 'total' colonization in a root system may not account for variance in old to new root portions, for example, nor variance in individual fine roots within one plant. Describing levels of colonization from a single sample-time in the host plant life cycle in the field is equally misleading as inter- and extra-radical AM fungal dynamics alter continually, considerably seasonally (Merryweather and Fitter 1998, Bohrer, Friese, and Amon 2004) and, as growth rates and levels of flux rate can be very high

(Schnepf, Roose, and Schweiger 2008), perhaps rapidly diurnally. It was decided initially to record only presence/absence of AM fungal organs in the observed root-segment samples, but the assessment method was later revised to a more accurate technique described by Biermann and Linderman (1981) where fine-root sub-samples from collective roots at a minimum of 25 segment percentage colonization assessments sample^{-1}.

The binocular microscope used throughout the study was an Olympus SZ 61 10 x 4.5 zoom stereo-microscope and the compound microscope (40x, 100x, 400x and 1000x oil-immersion) an Olympus BX 41. Micrographs were imaged by an Olympus U-CMAD 3 DP12 digital camera and were not digitally edited. Climate data were obtained from India Meteorological Dept., Mumbai.

CHAPTER 3

A TRANSECT SURVEY OF THE DUNE SYSTEM

Plate 3.1. The dune system**: a**) looking SW from the banyan tree, **b**) the transect line from the top of the foredunes, **c**) St. 1 and 2, **d**) foredunes from the beach.

3.1. Introduction

A number of studies of the distribution and abundance of AM fungal spores in coastal and arid psammite habitats are reported in the literature. Roda, Díaz, and Torres (2008), for example, found reducing numbers of spores on a salinity gradient in a salt marsh on the Alicante coast of Spain. Bai *et al*. (2009) described significant correlation of spores with soil

organic carbon (SOC) in rhizosphere soils of *Astragalus adsurgens* Pall. (Leguminaceae), a milkvetch in arid and semi-arid sandlands in northwestern China. D'Souza and Rodrigues (2013) concluded season and host co-affected spore density in their study in three mangrove species on the Goa coast. Few transect studies of primary dune systems have included AM spore abundance as a survey parameter. The dune system studied (Plate 3.1), although affected by short- and long-term perturbations consistent with coastal primary systems, e.g. variable topography due to wind action (Anthony and Orford 2002), fluctuation in water table (Kocurek *et al*. 1992), plant invasion (Alvarez and Cushman 2002), and advance and retreat of barrier foredunes before the sea (Hesp 1989), had previously been visually assessed to maintain a plant community biomass annual-cycle persistence overall that may equate to little variation in system-function stability. It was this observation along with that of a plant zonation pattern across the system that had prompted the transect-site selection.

The fungal phylum Glomeromycota, amongst other beneficial functions listed above, facilitates plant access to soil nutrients *via* an extensive mycelial network or 'web' that forages beyond rhizospheres (Helgason *et al*. 1998), enhancing uptake of P (Bolan 1991), N (Hodge, Campbell, and Fitter 2001) and micronutrients (Clark and Zeto 2000). The more extensive and persistant the web, the greater is the potential for nutrient sequestration. Where there are no extremes in precipitation, soil moisture and soil temperature during annual cycles such as in many temperate regions, a proportion of the web, and including root fragment MIP, retains viability to re-inoculate and colonize new roots during and in subsequent growing seasons (Smith and Read 2008). In this dry-tropical system, however, where hyphal inoculum potential is severely affected by loss of monsoon vegetative cover and desiccation by high soil temperatures (Koske *et al*. 2008), sporulation could play a significant survival

role in the annual cycle where spores better withstand the hot and dry sands environmental conditions between monsoons.

Thus an empirical record of AM genera and/or species spores prior to and after the rainy season may be a realistic assessment of AM fungal demography and abundance in the habitat community. The supposition is supported by evidence of similarity in AM species diversity assessed by molecular analysis compared with morphological identification of spore abundance presented by Clapp *et al*. (1995) and Helgason, Fitter, and Young (1999), both groups working with *Hyacinthoides non-scripta* (L.) Chouard *ex* Rothm. (bluebell) in the field.

Soil nutrients in natural ecosystems are spatially discrete at the macro-, meso- and microscales (Hodge 2005). In primary coastal dunes the distribution of deficient nutrients is as patchy (Olsson, Jakobsen, and Wallander 2002) as is plants distribution (Davy and Figueroa 1993, Jackson and Caldwell 1993, Hodge 2005). It was considered that if any pattern in edaphic factors were detected it may show similarity to or association with either plants demography or AM spores abundance data.

Plants, AM fungi, and soil edaphic factors are a tri-partite interactive system, each reciprocally affecting the other. Plant establishment stabilizes mobile sand grains, maintaining the process of coastal dune system geomorphology (Sloss, Shepherd, and Hesp 2012). Plants (Johnson *et al*. 2003) and soils (Read 2002) are drivers of AM fungi. In turn AM fungi mycelium distribute (and pool) photosynthate carbon throughout the hyphosphere stimulating soil respiration (Finlay 2008), actively enhance soil-crumb structure (Rillig 2004), and facilitate plant nutrition. Edaphic factors are intrinsically involved in plant species community composition, and there is strong evidence also of the involvement of AM fungi

(van der Heijden *et al*. 1998). The phyto- and myco-sociological relationships between the three in the natural environment are complex.

It was considered that gradients in edaphic factors might be detected along the transect line from front-foredune to the furthest point inland in the system during the monsoon growing season. It was anticipated there may be several; pH, salinity, ammomium-N, perhaps organic matter (OM) and sand-grain size (Maun 2009). Zonation in dominant plant species was visually obvious. As most, if not all, of the plant species are known to be mycorrhizal (Rodrigues and Jaiswal 2001, Beena *et al*. 2000), AM fungal spore dynamics may correlate with either feature, and dominant spore abundance indicate nutrient-function efficiency.

Other than recent additional plantings of coconut (*Cocos nuciferus* L.) and cashew (*Anacardium occidentale* L.), the extant plant community in the dune system chosen for the study is possibly little changed since establishment after the last seismic coastal-uplift event >8000 years ago (Rao and Wagle 1997). It is a 'near-stable' primary system, at least within the confines of short-term cyclic environmental perturbations. It is thus reasonable to assume that the AM fungal community is also near stable, that overall system nutritional function is more or less constant from year to year, and that species richness may have altered little in recent times. The assumption has further support in evidence presented by Kiers *et al.* (2011) that nutrient exchange control is both top-down and bottom-up and that "partners offering the best rate of exchange are rewarded", and Johnson *et al.* (2010) recently suggested the primary driver of local adaptation of AM fungi is edaphic resource availability. Nutrient-function inefficient AM fungal species, during the development and maintenance of this nutrient deficient ecosystem, would probably not attain a dominant community position. Thus by inference the dominant AM species may be those most nutrient-function efficient. Despite evidences of variation in AM fungal species temporal and spatial sporulation patterns (Bever

et al. 1996, Pringle and Bever 2002, Rosendahl and Stukenbrock 2004), empirical analysis of AM spores pre- and post-monsoon at genus and/or species level may prove dominance and, by the arguments presented, realistically indicate nutrient-function fitness.

The plant species in the system do not rely totally upon benefits gained through mycorrhization however. Each has anatomically or physiologically adapted to the environmental hostilities of the ecosystem. *I. pes-caprae*, for example, is semi-succulent, allowing low photosynthetic flux-rates required to maintain fitness. Large tap roots penetrate deeply to contact ground water (Ripley and Pammenter 2004). *Spinifex littoreus* has tough, leathery, narrow leaves enabling the species to withstand the rigours of salt spray wind-blown up and over the dune ridge, where it is generally restricted (Maun 2004). All of the perennial grass species have narrow, vertically oriented leaves, a strategy that preserves leaf boundary layer, and reduces radiant energy dissipation (Harper 1977).

The hypothesis is that survey of plant community, AM fungal spore community, and soil chemistry along an interrupted belt transect in a primary coastal dune system will distinguish any common patterns between the three, and may indicate functional attributes of AM fungi, particularly nutrient-function efficiency.

3.2. Objectives

- To derive empirical data of a) the observed plants zonation, b) AM fungal spore community characteristics, and c) spatial and temporal edaphic factors in rhizosphere soils along the transect.
- To determine plant community pattern, evaluate temporal and spatial soil nutrient statuses and assess spore density and diversity at genus and species levels.
- To statistically compare the datasets and ascertain which might be driving the relationships between the three.
- To extrapolate any suggestion of AM fungal role-play, particularly nutrient function, where spore abundance variation may be indicative of fitness of taxa.
- To assess whether there is empirical evidence that spore abundance is relative to nutrient-function efficiency, or there is indication of further experimental procedure that may test the thesis hypothesis.

3.3. Materials and methods

Field study of plant abundance, and edaphic and environmental factors, was carried out during monsoon 2010. The total length of the selected transect described in General Materials and Methods was measured and Stations (St.) 1-7 established where there was visual impression of differing plant zones, the distance of each from St. 1 (5 m) recorded (Fig. 2.2).

A grid-quadrat survey of plants (see General Materials and Methods for procedure) was conducted to assess plant frequency when communities were visually assessed at or about seasonal maximum biomass (20.9.10). The soil sampling methods followed are described in General Materials and Methods. Samples for soil characteristics assessment were taken on five separate occasions, the first before monsoon rains (date 6.6.10: day 0), three during rains (day 20, 67, 129), and the last after cessation (day 189) (Series 1-5). The chemical analysis protocols and procedures followed are described in General Materials and Methods. Sand-grain particle size was measured by passing air-dried 50 g samples (n = 3) from each station through nested 1003, 500, 250 and 52 µm sieves (Cheetham *et al*. 2008) after first removing large OM fraction above 2 mm. Soil-water content samples (n = 5 from each station) were extracted during the rainy season, 1 hour and 4 days after rain, to a 20 cm depth and percentage determined gravimetrically for each station from homogenized samples after drying at 105ºC for 6 h. Silt/clay fraction was assessed by water-column suspension, the hydrometer method (Singh, Chhonkar, and Dwivedi 2005), without replication. Further soil Na concentration assessment was made across the transect stations on 27.2.12 and 25.5.12, five samples from each station, homogenized.

Samples for AM fungal spore abundance and diversity evaluation were extracted on two occasions, post-monsoon (4.12.11) after plant community had desiccated and spore abundance is likely to be near maximum (see Fig. 1.3, AM fungi life-cycle), identified to

genus level, and pre-monsoon 25.5.12, before the viable spores remaining after the dry season germinate, identified to species level. Spores were recovered from 3x 50 g sub-samples of the rhizosphere soils. Spore integrity was carefully visually assessed before removal of healthy specimens from Whatman No.1 filter paper (see General Materials and Methods for procedures). Filter-paper root fragments were also examined for intraradical spores, their (few) numbers included in counts. Sporocarps were classified as single units. Root colonization was confirmed by the Phillips and Hayman (1970) method. The procedures followed are described in General Materials and Methods. Simpson's Index of Diversity (1-D) was used as a measure of species diversities over distance in the plant community, and in spores communities at both genus and species levels.

Statistics

Histograms and line graphs were drawn in Excel 2007. Scatterplots were drawn in Excel and in Minitab 16. Gradient analysis, Analysis of Variance (ANOVA) where pairwise comparisons were made by Tukey's Honestly Significant Difference (HSD) test at $P = <0.05$ (Hsu 1996), and Principal Components Analysis (PCA) were carried out in Minitab 16. Correspondence Analysis (CA), Canonical Correspondence Analyses (CCA) and Detrended Canonical Correspondence Analysis (DCCA) were carried out in the CANOCO v4.51 package. The length of the longest gradient from DCCA was 2.7, suggesting there was no need for a DCCA over a CCA (Pers. Comm. Prof. T.H. Sparks). Using the nomenclature of CANOCO, the plant species x location (i.e. distance of the transect stations from MH-WM) data matrix was compared with the spores x location data matrix (as "environmental variables") and soil chemistry x location data matrix (as "supplementary environmental variables"). Plant species data were log(x+1) transformed and down-weighted for rare

species, ensuring that the ordination was not dominated by the most common plant species, nor overly influenced by the rare species.

Pearson's correlation coefficient applies to all references to correlation in the text. A correlation matrix of plants and soil factors data was initially constructed in WebAgris 2.0 package, Indian Council for Agricultural Research (ICAR). Pearson's was also used as a test where CCA indicated association.

Simpson's index of diversity (1-D) was calculated for plants, and for AM fungal spores at both genus and species level, at each station and over the transect.

3.4. Results

3.4.1. Plant distribution

The plant species encountered on the transect were prostrate, patchy in distribution (Plate 3.1a, b, c), and diversity limited where 1-D was <0.600 in St. 2 and 4 only, and 0.816 over the whole transect (Table 3.1). All species but one proved to be consistently AM mycotrophic and, in such a hostile, nutrient deficient environment, probably obligately so.

Table 3.1. Plant species frequency in 7 stations on a transect line in a Goa (India) primary coastal dune system.

	St. 1	St. 2	St. 3	St. 4	St. 5	St.6	St.7
Distance from MH-WM (m)	5	20	35	65	101	138	175
Species	**%**	**%**	**%**	**%**	**%**	**%**	**%**
Spinifex littoreus (Burm.f.) Merr. (Poaceae) (P;C)	13.75 (±9.5)	37.50 (±14.1)	-	-	-	-	-
Ipomoea pes-caprae (L.) R.Br. (Convolvulaceae) (P;C)	56.25 (±9.4)	2.50	10.42	-	-	-	-
Digitaria adscendens (H.B.K.) (Poaceae) (P;C)	12.50	1.25	-	1.25	-	-	-
Launaea fallax Jaub. & Spach (Asteraceae) (B/P; C)	2.50	-	-	1.25	6.25	1.25	-
Cyperus arenarius Retz. (Cyperaceae) (P)	1.25	6.25	21.25 (±2.8)	48.75 (±8.9)	3.75	15.00 (±7.2)	-
Digitaria stricta Roth. (Poaceae) (A)	-	-	-	-	-	2.50	-
Waltheria indica L. (Sterculiaceae) (P)	-	-	-	8.75	51.25 (±15.6)	15.00 (±9.5)	-
Dactyloctenium aegyptium L. (Poaceae) (A;NR)	-	-	-	-	12.50	20.00 (±8.7)	21.25 (±5.7)
Zoysia matrella (L.) Merr. (Poaceae) (P;C)	-	-	-	-	53.75 (±10.9)	55.00 (±17.1)	28.75 (±13.0)
Panicum repens L. (Poaceae) (P;C)	-	-	-	-	5.00	-	-
Alysicarpus vaginalis L. (Fabaceae) (P;NR)	-	-	-	-	-	12.50	27.50 (±17.9)
Perotis indica L. (Poaceae) (A)	-	-	-	-	-	-	1.25
Ischaemum indicum (Houtt.) Merr. (Poaceae) (P)	8.75	61.25 (±4.7)	71.25 (±5.78)	68.75 (±15.0)	33.75 (±16.5)	75.00 (±14.8)	100.00
Simpson's Index of Diversity (1-D)	**0.646**	**0.595**	**0.871**	**0.596**	**0.745**	**0.774**	**0.646**

Samples SD in parenthesis. Overall Simpson's Index of Diversity (1-D) = 0.816. A = annual, B = biennial, C = clonal, MH-WM = Mean High-Water Mark, NR = nodal rooting, P = perennial.

Quadrat survey data supported the visual impression of plant zonation (Table 3.1). *Ischaemum indicum* was ubiquitous at variable density, low in the front-foredune slope and St. 5, dominant in four of the remaining five stations and a 100% plant frequency recorded in St. 7. *Ipomoea pes-caprae* and *Spinifex littoreus* were recorded only in the foredunes and the perennial grass *Digitaria stricta*, at low levels, only in St. 6. *Cyperus arenarius* (the only consistently non-mycorrhizal species) was found at relatively high density in St. 4 only but was represented in all stations except the last. *Waltheria indica* density was high only in St. 5, the species restricted to St. 4-6. *Dactyloctenium aegyptium* and *Zoysia matrella* were restricted to the last three stations (St. 5-7), and the leguminous forb *Alysicarpus vaginalis* appeared only in the two stations furthest from MH-WM (St. 6 and 7). The correlation matrix indicated significant positive correlations between frequency of *I. pes-caprae* and *Digitaria adscendens* ($r = 0.970$; $P = 0.003$) in St. 1 to 4, between *Launaea fallax* and *W. indica* ($r = 0.905$; $P = 0.017$) in St. 4 to 6, and between *D. aegyptium* and *Z. matrella* ($r = 0.858$; $P = 0.031$) in St. 5 to 7. CCA (Fig. 3.1) explains 50.4% and 20.5% (total 70.9%) of the plant species : station location relationship on the first two axes respectively. Ten of the 13 species recorded are perennials, seven clonal in habit (Table 1.1). All eight grass species are C_4 (Waller and Lewis 1989), five are clonal perennials, and one of the three annuals, *D. aegyptium*, nodal rooting. All perennials but *I. indicum* were rooted deeply, particularly genets (see Glossary) of *I. pes-caprae* (Duvall 1992) in the foredunes (Plate 3.2).

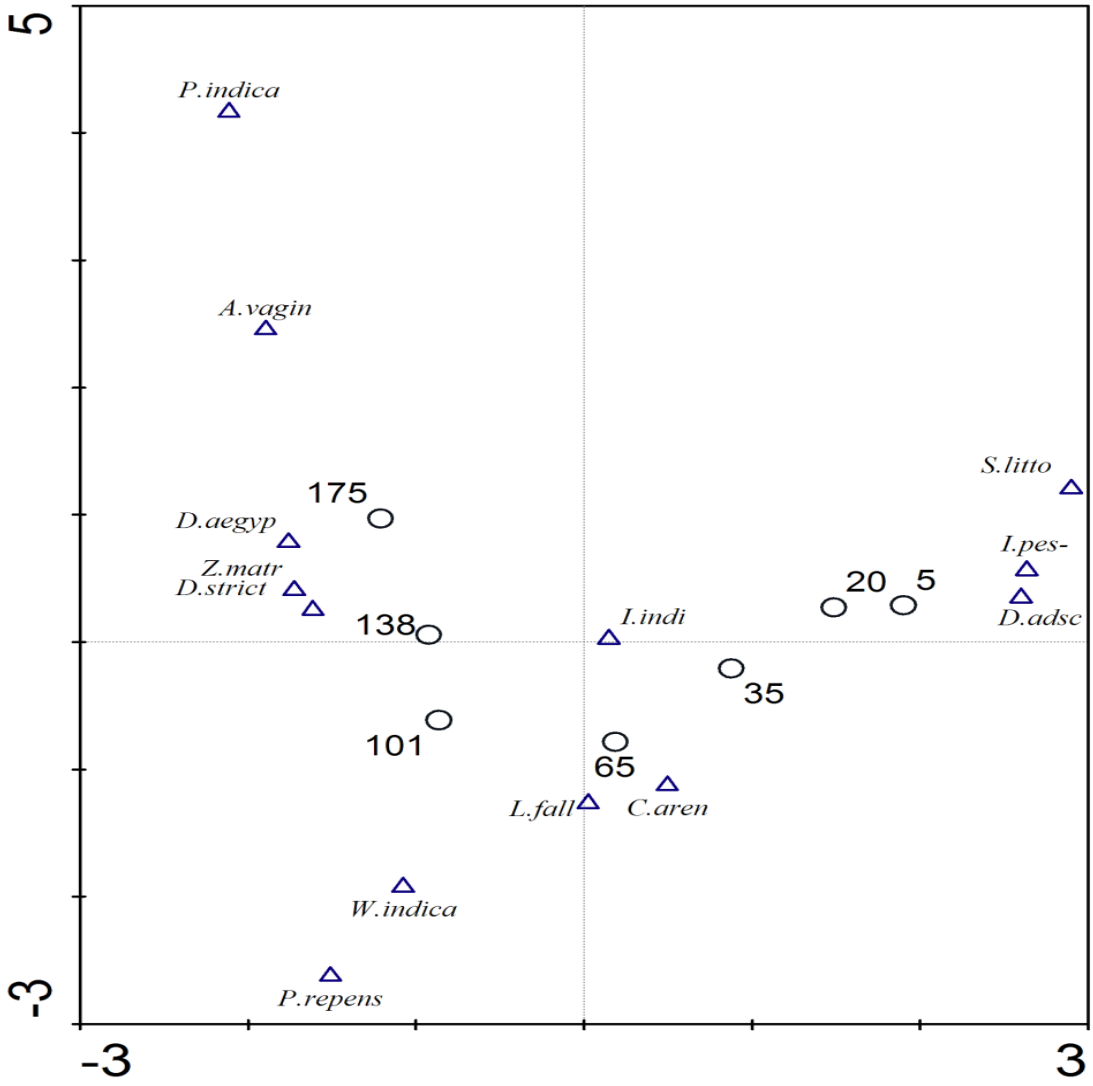


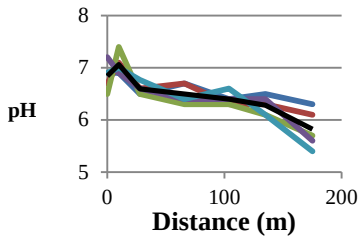
Fig. 3.1. CCA biplot showing ordination of plant species (triangles) and Station 1-7 locations in m from MH-WM (circles). For full list of species names see **Table 1**. The two axes jointly explain 70.9% of the variation.

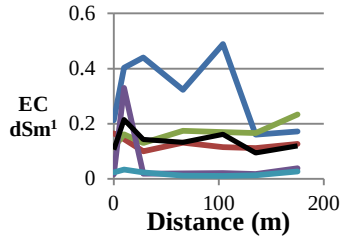
Plate 3.2. Deep roots of *I. pes-caprae* exposed where high tides had washed away the dune face *ca* 60 m south of the study site.

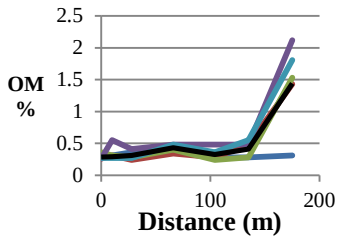
3.4.2. Soil characteristics

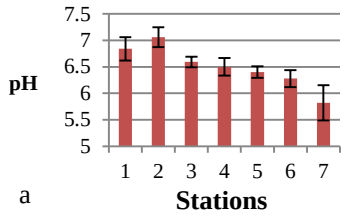
3.4.2.1. Spatial scale

Rhizosphere soil chemistry analyses SD indicate variation in the 7 station locations along the transect. Mean pH (Fig. 3.2a) followed a general trend toward acidity after an initial slight increase in St. 2 on the crest of the foredunes, and decreased sharply in the last station where variance was greatest. There was an overall increase in mean acidity by an order of almost one magnitude (range pH 7.2-5.4). A transect-wide pH gradient was indicated across the system (Fig. 3.3). There were no other similarly clear gradients.

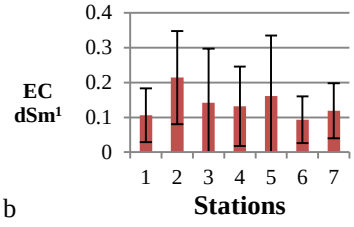

pH
8
7
6
5
0
100
200
Distance (m)


EC
dSm¹
0.6
0.4
0.2
0
0
100
200
Distance (m)

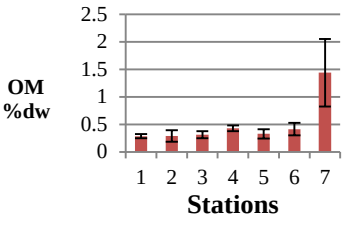

OM
%
2.5
2
1.5
1
0.5
0
0
100
200
Distance (m)


pH
7.5
7
6.5
6
5.5
5
1 2 3 4 5 6 7
Stations

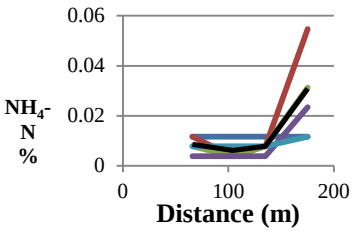
a

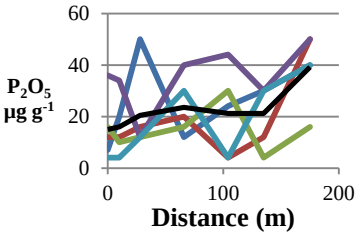

EC
dSm¹
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7
Stations

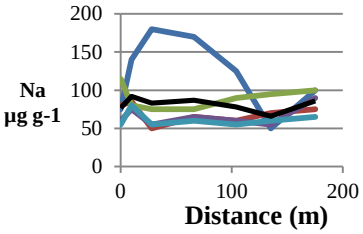
b

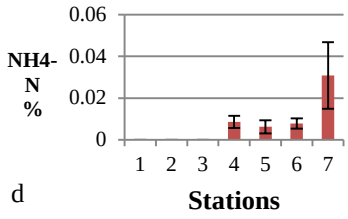

OM
%dw
2.5
2
1.5
1
0.5
0
1 2 3 4 5 6 7
Stations

c

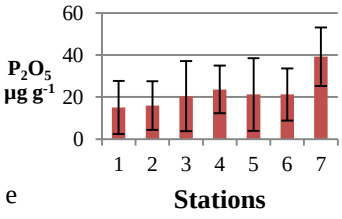

NH4-
N
%
0.06
0.04
0.02
0
0
100
200
Distance (m)


P2O5
µg g-1
60
40
20
0
0
100
200
Distance (m)

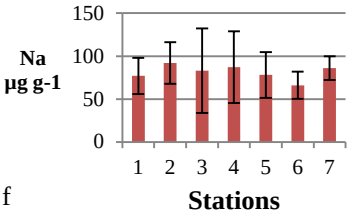

Na
µg g-1
200
150
100
50
0
0
100
200
Distance (m)


NH4-
N
%
0.06
0.04
0.02
0
1 2 3 4 5 6 7
Stations

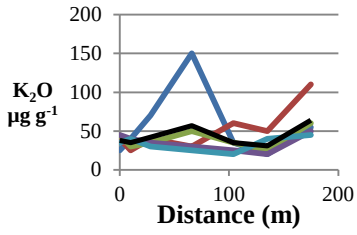
d

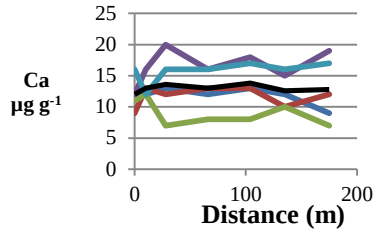

P2O5
µg g-1
60
40
20
0
1 2 3 4 5 6 7
Stations

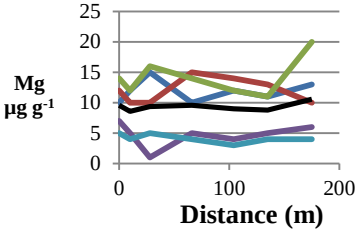
e

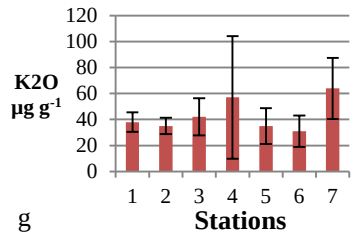

Na
µg g-1
150
100
50
0
1 2 3 4 5 6 7
Stations

f

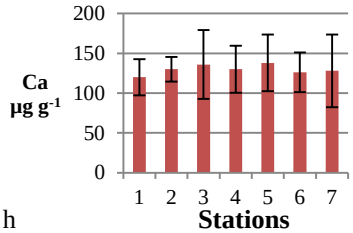

K2O
µg g-1
200
150
100
50
0
0
100
200
Distance (m)


Ca
µg g-1
25
20
15
10
5
0
0
100
200
Distance (m)

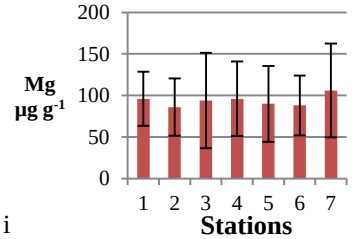

Mg
µg g-1
25
20
15
10
5
0
0
100
200
Distance (m)


K2O
µg g-1
120
100
80
60
40
20
0
1 2 3 4 5 6 7
Stations

g


Ca
µg g-1
200
150
100
50
0
1 2 3 4 5 6 7
Stations

h


Mg
µg g-1
200
150
100
50
0
1 2 3 4 5 6 7
Stations

i

Fig. 3.2. Scattergrams over distance in m from MH-WM, and corresponding Stations Nos. 1-7 histograms of soil chemistry data. Error bars represent SD about the time-sequence means. The means data were used in statistical analyses. Range is referred to in the text where it exceeded SD.
Distances: (St. 1-7), 5, 20, 35, 65, 101, 138 and 175 m from MH-WM.
Chronosequence: _____0d : _____20d : _____67d : _____129d : _____189d : _____ Means.
A 2-way ANOVA analysis indicates a spatial (i.e. distance from MH-WM) effect on pH (F = 15.98; *P* = 0.001), OM (F = 12.96; *P* = 0.001) and NH_4-N (F = 8.83; *P* = 0.001), and a time effect on EC (F = 13.93; *P* = 0.001), P_2O_5 (F = 3.34; *P* = 0.026), Ca (F = 16.34; *P* = ,0.001), Mg (F = 31.65; *P* = ,0.001) and Na (F = 6.47; *P* = 0.001). There was no significant effect of either distance or time upon K_2O.

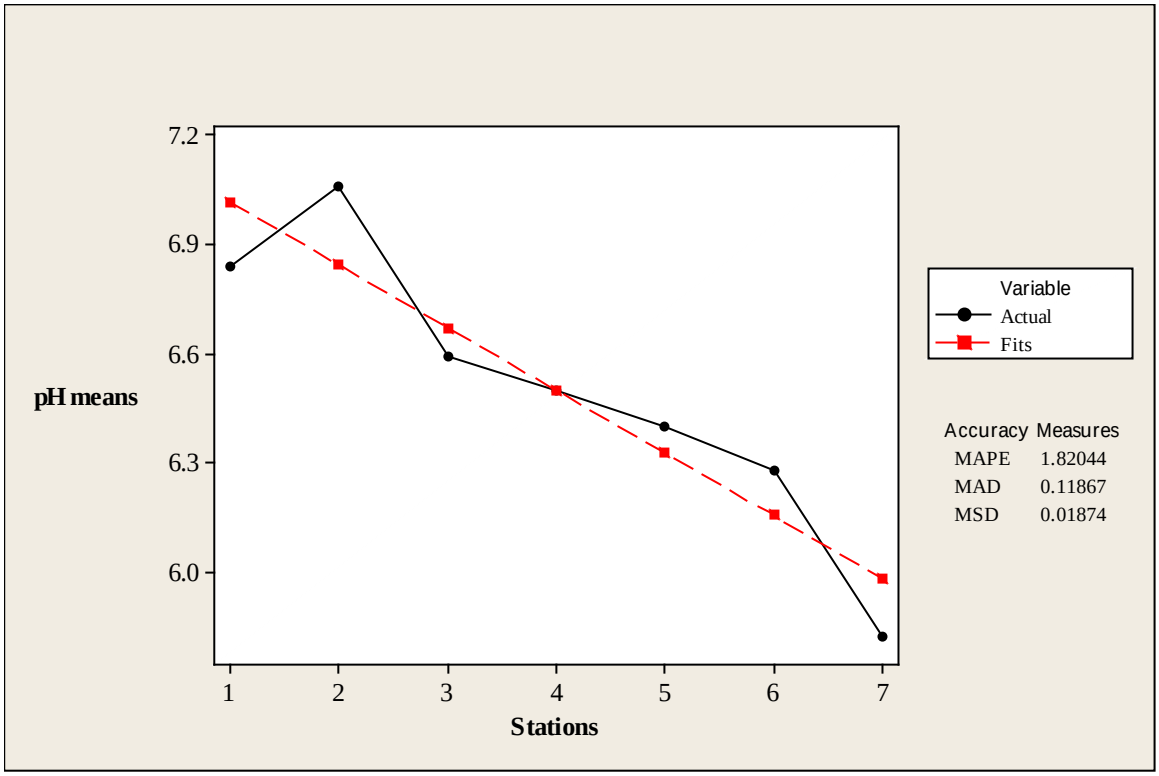


Fig. 3.3. Gradient analysis of pH means along the transect. Slope Yt = 7.187 – 0.1712929*t, where Y = pH means of data recorded over the sample time points (Series 1-5) and t = locations along the transect. The mean absolute % error (MAPE), mean absolute deviation (MAD) and mean squared deviation (MSD) accuracy measures are all small, indicating a robust fit (Hobai 2009).

Canonical Correspondance Analysis (CCA) (Fig. 3.4) indicates a transect-wide negative association between pH and OM (r = -0.813; *P* = 0.047) and between pH and P_2O_5 (r = -0.887; *P* = 0.022). Correlation between OM and pH in the partial (St. 1-3) transect is weak (r = -0.593), but in the partial (St. 4-7) transect there was a significant negative relationship (r = -0.948; *P* = 0.007). EC (Fig. 3.2b) varied throughout the monsoon, particularly in St. 2-5 where maximum range 0.49 dS m^{-1} exceeded SD in the period prior to the onset of monsoon rains. OM percentage dry weight (Fig. 3.2c) was <0.5% throughout the time period in St. 1-6, and up to 4 x higher (range maximum 2.12%) in St. 7 after the onset of rain (see Fig. 3.9 for rainfall details).

Further CCA (Fig. 3.4) indicated strong positive association of OM with P_2O_5 (r = 0.959; *P* = 0.005), and weak association with Mg (r = 0.811; *P* = 0.048). A pattern similar to OM was shown in a partial dataset of NH_4-N (Fig. 3.2d), low and even distribution in St. 4-6 with sharp increase in St. 7 where there was a reduction on cessation of rains. Plant available P_2O_5 (Fig. 3.2e) means and SD varied widely through the time period in all stations. The greatest variation in Na levels (Fig. 3.2f) was in St. 3, on the back-slope of the foredunes and in St. 4, high levels recorded prior to rains (up to range maximum 180 µg g^{-1}) and rapidly leached to comparatively low levels (<55 µg g^{-1}) after the onset of monsoon rain. There was no significant correlation of mean Na with EC over the transect length. K_2O (Fig. 3.2g) showed greatest variance in St. 4 where pre-monsoon maximum range was singularly high at >150 µg g^{-1}, with less variation in St. 7. Canonical correspondence analysis (Fig. 3.4) indicated there was association between K_2O and Mg, confirmed by correlation (r = 0.877; *P* = 0.025). A weak and non-significant negative correlation was indicated between Ca and Mg (Fig. 3.2h, i) and there was no significant correlation of Ca with any other factor. Canonical correspondence analysis (Fig. 3.4) confirms there are no soil chemistry patterns similar to plant community zonation structure over the length of the transect.

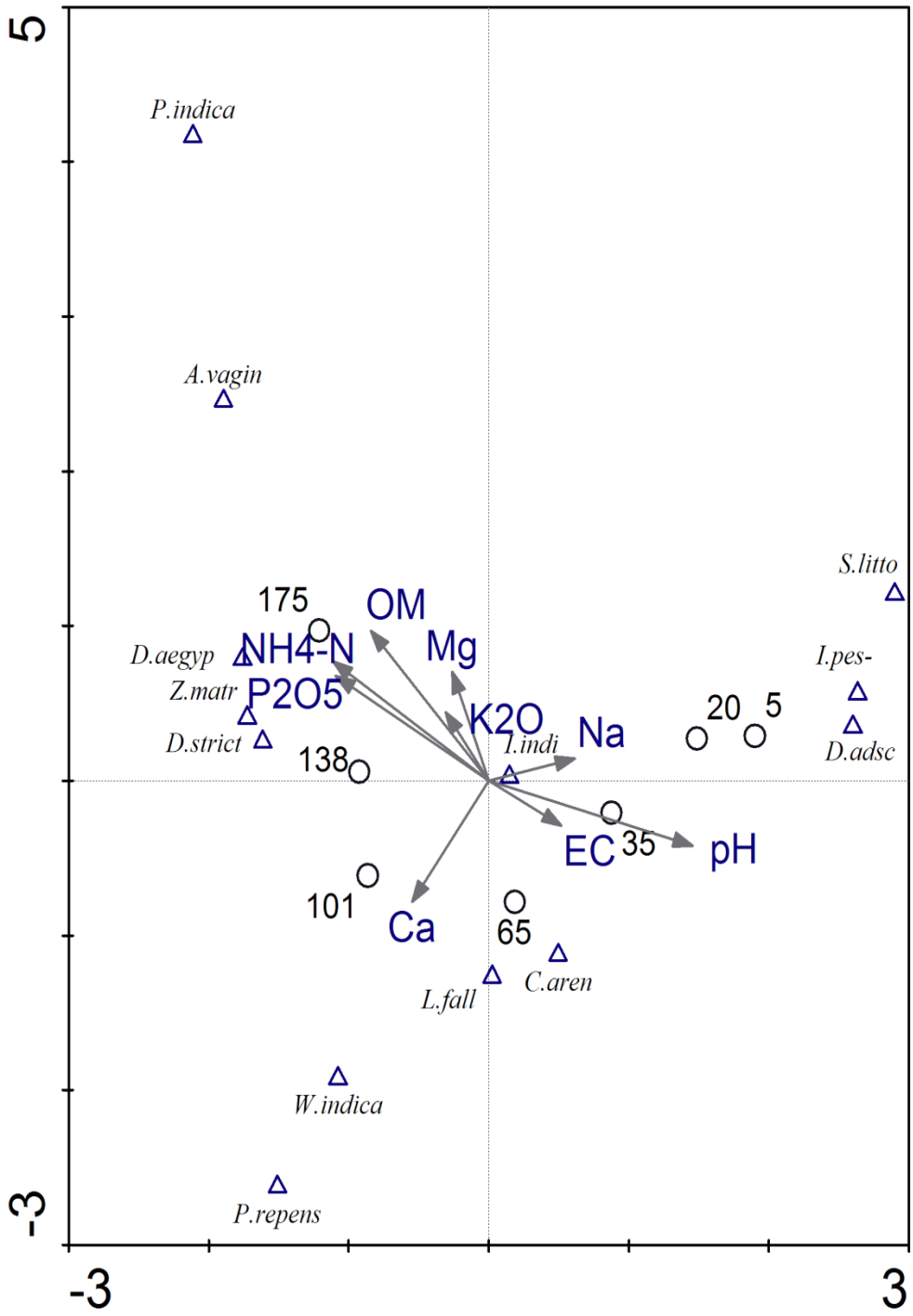


Fig. 3.4. CCA triplot showing ordination of plant species (triangles), transect stations locations (circles) and mean soil chemistry data of Series 1-5 (arrows). For full list of species names see **Table 1**. The two axes jointly explain 70.9% of the variation.

3.4.2.2. Temporal scale

From the correlation matrix, the analyses of Series 1-5 soil chemical characteristics, that are indication of change over the time period, showed significant correlation in pH : EC (r = 0.992; *P* = 0.026), significant correlation in pH : K_2O (r = 0.929; *P* = 0.022), significant negative correlation in EC : OM (r = -0.943; *P* = 0.016), significant correlation in EC : Na (r = 0.962; *P* = 0.009), weak and non-significant correlation between EC and K_2O (r = 0.876; *P* = 0.052), significant negative correlation in Ca : Mg (r = -0.975; *P* = 0.005), significant correlation between NH_4-N and K_2O (r = 0.920; *P* = 0.027), negative correlation between OM and Na (r = -0.853; *P* = 0.066) that is not significant but may indicate Na is not complexed in OM, and significant negative correlation between OM and K_2O (r = -0.878; *P* = 0.05) that may indicate K_2O is not complexed with OM. The data are presented in Table 3.2.

Table 3.2. Correlations of temporal variations in time points 1-5 soil chemistry analyses, *P* = <0.05.

	r	*P*	
pH : EC	0.992	0.026	
pH : K_2O	0.929	0.022	
EC : OM	-0.943	0.016	
EC : Na	0.962	0.009	
EC : K_2O	0.876	0.052	n/s
Ca : Mg	-0.975	0.005	
NH_4-N : K_2O	0.920	0.027	
OM : Na	-0.853	0.066	n/s
OM :K_2O	-0.878	0.050	

n/s = non-significant

ANOVA of the five factors affected by time in the Series 1-5, EC, P_2O_5, Ca, Mg and Na (sub-script **Fig. 3.2**), indicates: a) EC: Series 1 is significantly greater than (F = 8.07; *P* = 0.015), and Series 5 significantly less than, Series 2, 3 and 4; b) P_2O_5: Series 4 is

significantly greater than Series 3 (F = 13.61; P = 0.003), differences in the other three Series are non-significant; c) Ca: Series 4 and 5 are significantly greater than Series 1, 2 and 3 (F = 12.40; *P* = <0.001); d) Mg: Series 4 and 5 are significantly less (F = 35.37; *P* = <0.001) than Series 1,2 and 3; and e) Na: pre-monsoon Series 1 is significantly greater (F = 7.08; *P* = <0.001) than the four following series.

3.4.3. Silt/clay, soil-water and sand-grain particle size

From the correlation matrix, soil silt/clay fraction data (Fig. 3.5) was found to be significantly positively correlated with P_2O_5 (r = 0.860; *P* = 0.031), with NH_4-N (r = 0.966; *P*= 0.004), Mg (r = 0.925; *P* = 0.012), OM (r = 0.919; *P* = 0.014) and with K_2O (r = 0.883; *P* = 0.024) (Table 3.3).

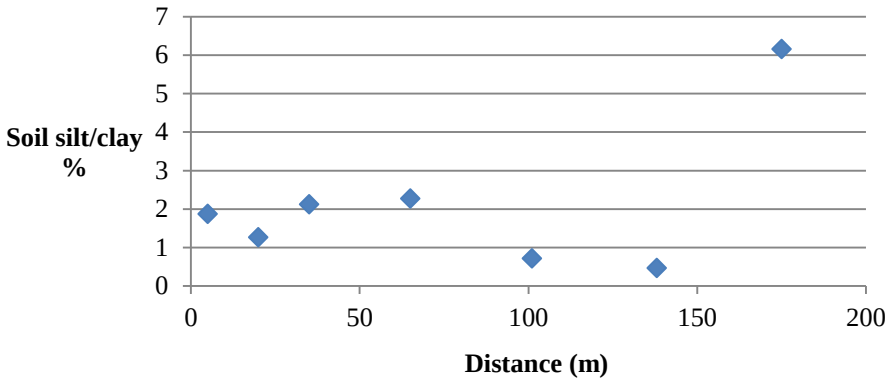


Fig. 3.5. Scatterplot of silt/clay fraction of transect soils.

Soil-water content assessment (Fig. 3.6) across the transect indicated higher levels of retention in the stations further inland. Correlation indicates significant similarity in the two datasets, 1 h after rain and 4 d after rain. There was significant correlation of both 1 h mean soil water content with OM (r = 0.738; *P* = 0.038) and 4 d mean soil water content with OM (r = 0.990; *P* = <0.001).

Table 3.3. Pearson's correlation between silt/clay, OM and mineral nutrients (n = 5).

	r	*P*
Silt/clay : OM % d.w.	0.919	0.014
Silt/clay : NH_4-N	0.966	0.004
OM % d.w. : P_2O_5	0.959	0.005
OM % d.w. : Mg	0.811	0.048
OM % d.w. : K_2O	0.769	0.064
Silt/clay : P_2O_5	0.860	0.031
Silt/clay : Mg	0.925	0.012
Silt/clay : K_2O	0.883	0.024

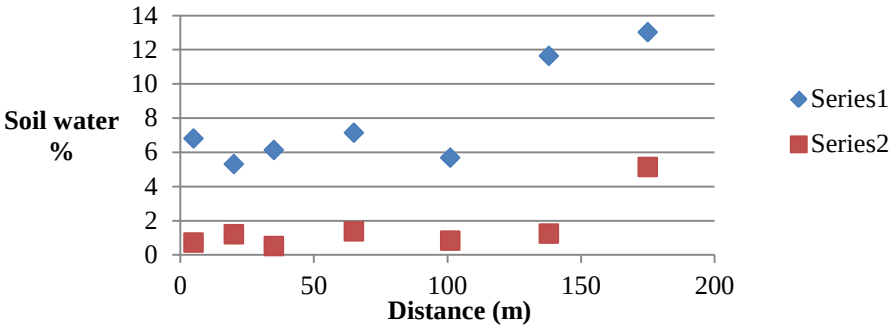


Fig. 3.6. Scatterplot of soil water retention in transect soils. Series1 = 1 h after rain, Series2 = 4 d after rain. ANOVA indicates sig. diff. (F = 23.79; *P* = <0.001). Correlation r = 0.765; *P* = 0.045.

A negative correlation was indicated between <52 µm sand-grain particle size data (Fig. 3.7) and soil water content (r = -0.861; *P* = 0.030), and a significant positive correlation between 250-500 µm sand-grain particles and soil water (r = 0.820; *P* = 0.045). The two sand grain fractions made up >97.5% of the matrix overall. There was no significant correlation found between rainfall (Fig. 3.8) and any other edaphic factor in the system.

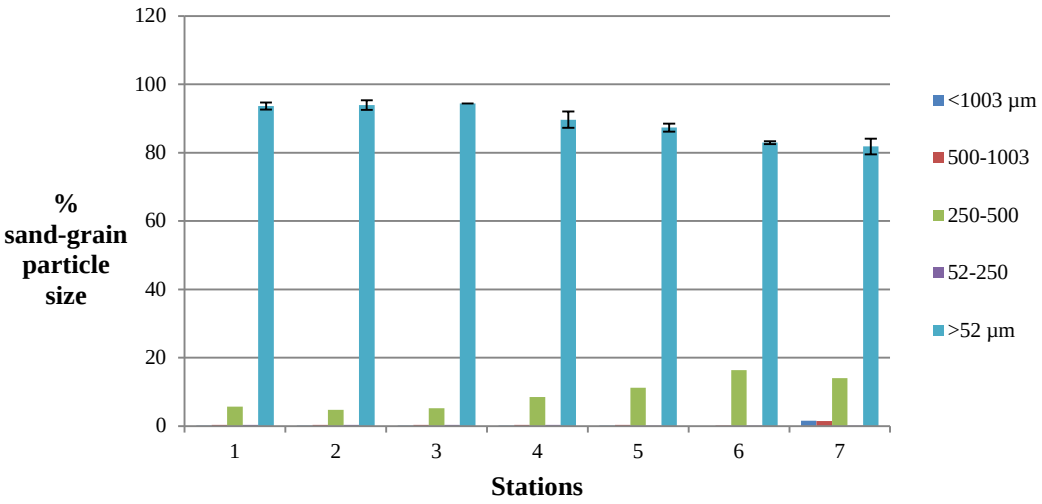


Fig. 3.7. Histogram of mean **s**oil sand-grain particle size on a transect in a Goa, India primary dune system. Error bars are SD of 3 replicates.

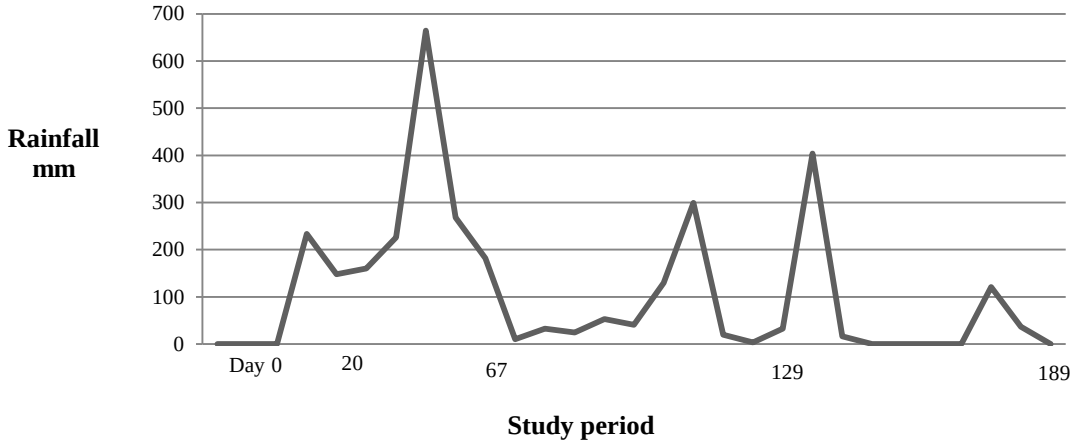


Fig. 3.8. Rainfall during periods between samples collection dates in Morjim (Goa) dunes, dataset at 7-day intervals.

Levels of Na concentrations in samples collected during the hot, dry months of February and May (Fig. 3.9) indicated significant correlation in transect pattern, the May dataset overall mean 30.2% lower than that recorded in February.

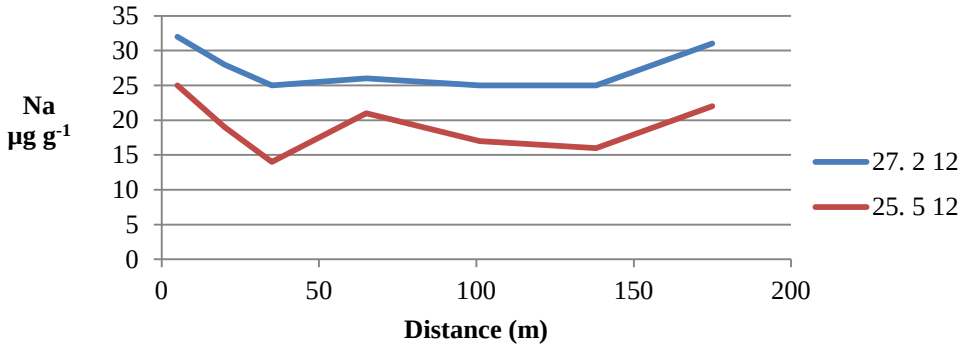


Fig. 3.9. Scatterplot of Na concentrations during the dry season. ANOVA indicates sig. diff. (F = 25.1; P = 0.001), and correlation sig. similarity (r = 0.872; *P* = 0.010), between the two.

3.4.4. Spores abundance, distribution and diversity

Overall, spores of 16 AM fungal species in four genera were extracted from rhizosphere soils in the transect. There were similar orders of rank abundance in the post- and pre-monsoon datasets at genus level (Fig. 3.10a, b), *Acaulospora* the highest, followed by Gigasporaceae, then *Glomus*. *Acaulospora* constituted 66.0% of the total in the post-monsoon set (4.12.11), 53.8% in the pre-monsoon set (25.5.12), Gigasporaceae 21.3% and 30.8% respectively, and *Glomus* 12.7% and 15.4%. *Acaulospora* spore numbers increased by 19.4% between datasets (the dry season), and Gigasporaceae and *Glomus* by 43.2% and 20.5%, respectively. *Acaulospora* and Gigasporaceae spore numbers fell sharply to the lowest levels on the transect at St. 7 in both datasets, *Glomus* taking a dominant position. 1-D of the overall post-monsoon line transect was 0.510, 0.592 in the following dataset (Fig. 3.10a, b). Two-way ANOVA indicates there is no significant influence of time, i.e. post- (4.12.11) to pre- (25.5.12) monsoon, or distance on Simpson's diversity.

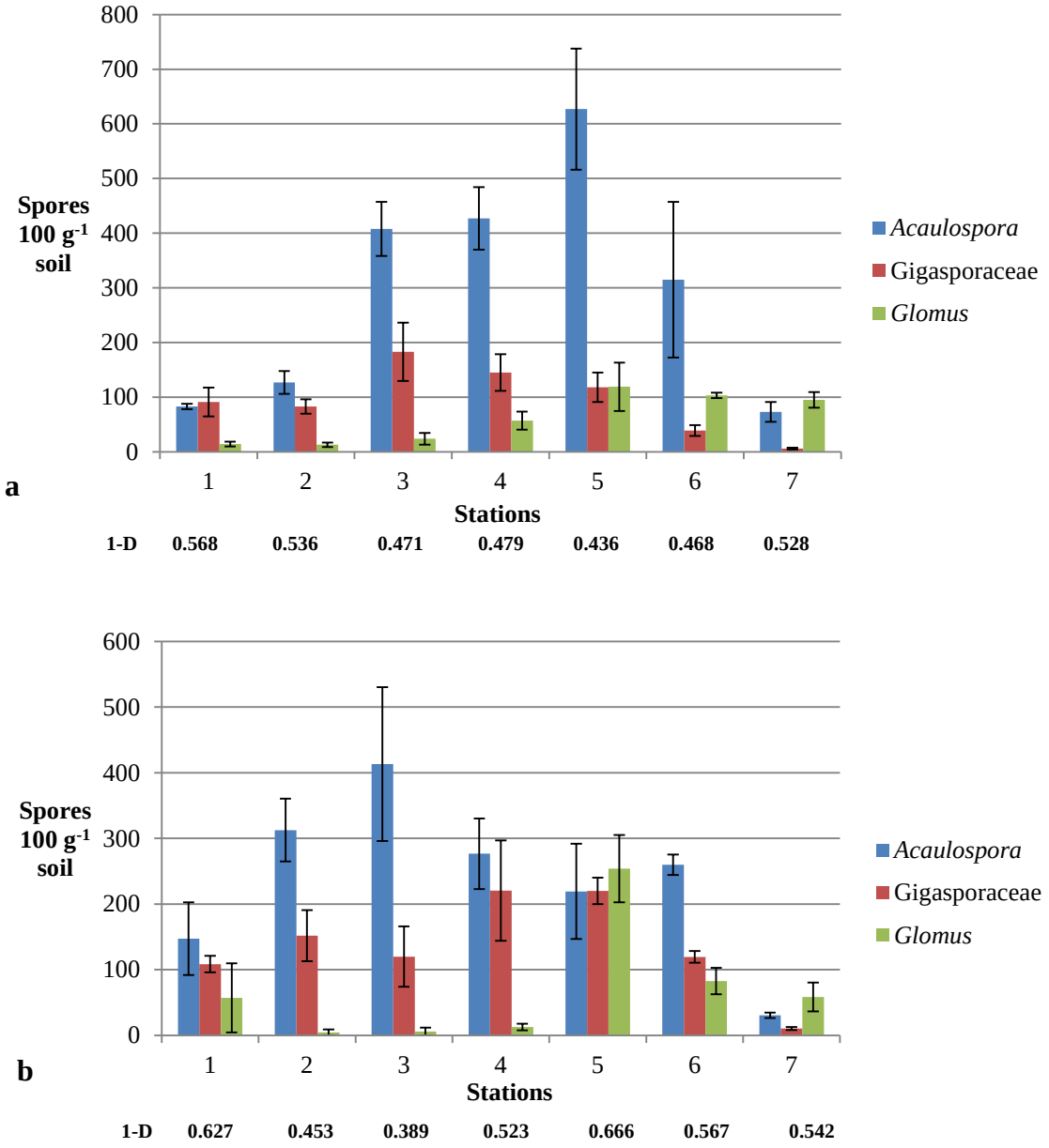


Fig. 3.10. Distributions of AM fungal genera spores in rhizosphere soils on a transect in Morjim (Goa) sand dunes. Collection dates were **a**) 4.12.11 post-monsoon, data representing AM population after the growing season; **b**) 25.5.12 pre-monsoon, data representing MIP for the following growing season. Diversity index (1-D) is shown for each station. Error bars represent SD.

Correlation over the entire transect between edaphic factors and post-monsoon (4.12.11) spores genus level data revealed significant negative correlation of pH with *Acaulospora* ($r = -0.862$; $P = 0.030$) only. Following the parting of the NH_4-N partial dataset (Fig.3.2d)

however, correlation of St. 1-3 OM datasets with *Acaulospora*, Gigasporaceae and *Glomus* indicated strongly significant (P = <0.002) positive correlations (r = 0.999, 0.977 and 0.975 respectively). Partial (St. 4-7) OM analysis indicated significant negative correlation with *Acaulospora* (r = -0.868; P = 0.028) and negative but non-significant correlation with Gigasporaceae and *Glomus.*

Principal components analysis (PCA) of spores at genus level in the two datasets versus plants distribution over the transect (Fig. 3.11) indicates *Glomus* spp. were present almost exclusively in the latter stations, *Acaulospora* spp. and Gigasporaceae more abundant in the earlier stations. An exchange in transect position of greatest abundance in *Acaulospora* and Gigaspoaceae was indicated in the transition from post-monsoon to pre-monsoon during the dry season.

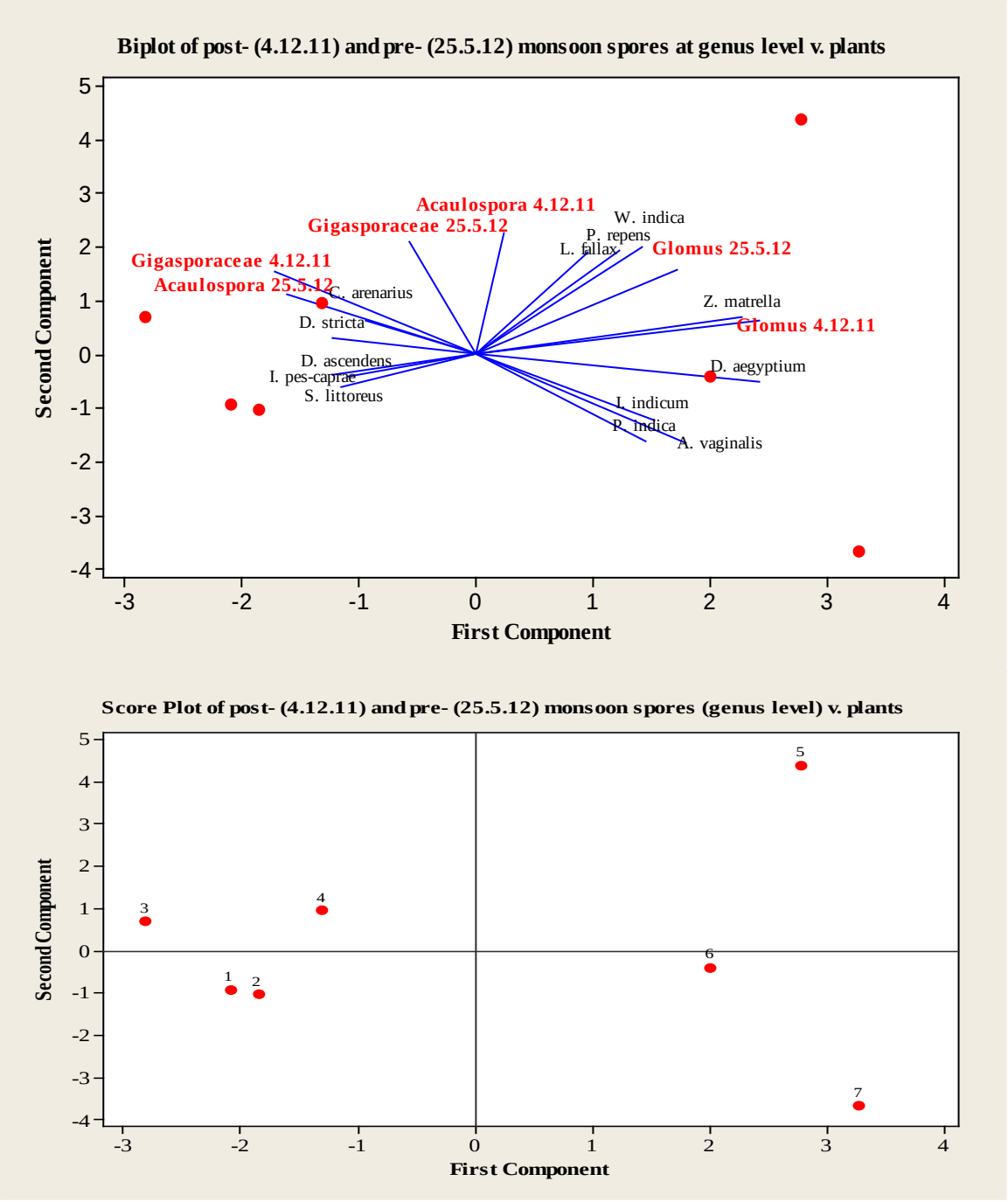
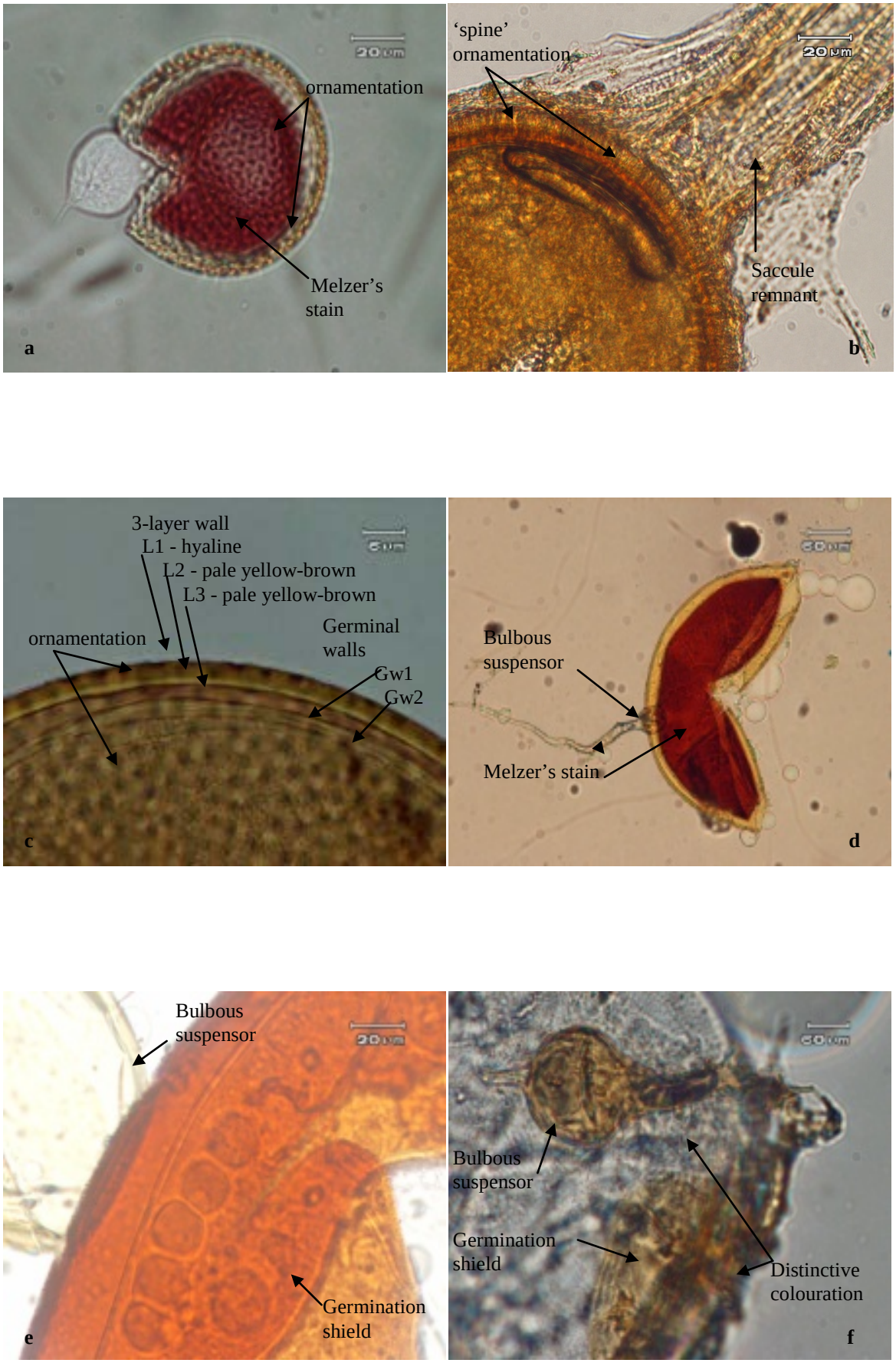


Fig. 3.11. PCA of spore diversity at genus level from pre- and post-monsoon datasets against plants distribution. Score Plot indicates Stations 1-7. For full list of plant species see **Table 3.1**. The two axes jointly explain 66.7% of the variation.

At species level 16 AM species were morphologically identified (see Plate 3.3 for micrographs of 10 of these species) in the pre-monsoon 25.5.12 dataset (Fig. 3.12). *Acaulospora spinosa* Walker & Trappe spores were the most numerous, 72.9% of all *Acaulospora* species and 39.7% of total spores, >99% of these recovered from St. 1 to St. 6, followed by *Gigaspora margarita* Becker & Hall, 63.9% of all Gigasporaceae species, 20.0% of total, 72.3% recovered from St 1 to St. 4. *A. scrobiculata* Trappe was 16.6% of *Acaulospora* spp.and 9.0% overall, *G. claroideum* Schenck & Smith 59.6% of *Glomus* and 8.5% of total, and *Scutellospora gregaria* (Schenck & Nicol) Walker & Sanders, 24.4% of all Gigasporaceae, 7.6% of total spores. *Glomus felinovii* was 21.3% of *Glomus*, 2.5% of total. These six species represent 87.4% of total spores recovered. The dominant genus at St. 7 was *Glomus*, the dominant species *G. albidum* Walker & Rhodes, albeit at low abundance, un-recorded in the previous stations. Correlation analysis between AM fungal species spores over the line transect indicated significant (r = 0.996; P = <0.0001) association between *S. heterogama* (Nicol. & Gerd) Walker & Sanders and *G. claroideum* only. There were no significant negative correlations to suggest direct competition. 1-D was 0.779 over the whole transect, only St. 4 greater than that at 0.791 (Fig. 3.12). Relative abundance of AM fungal species in rank order is shown in Fig. 3.13. Figure 3.14 shows SD of 12 of the 16 species recovered, >98% of the total recovery.


ornamentation
Melzer's stain
a
'spine' ornamentation
Saccule remnant
b
3-layer wall
L1 - hyaline
L2 - pale yellow-brown
L3 - pale yellow-brown
ornamentation
Germinal walls
Gw1
Gw2
c
Bulbous suspensor
Melzer's stain
d
Bulbous suspensor
Germination shield
e
Bulbous suspensor
Germination shield
Distinctive colouration
f

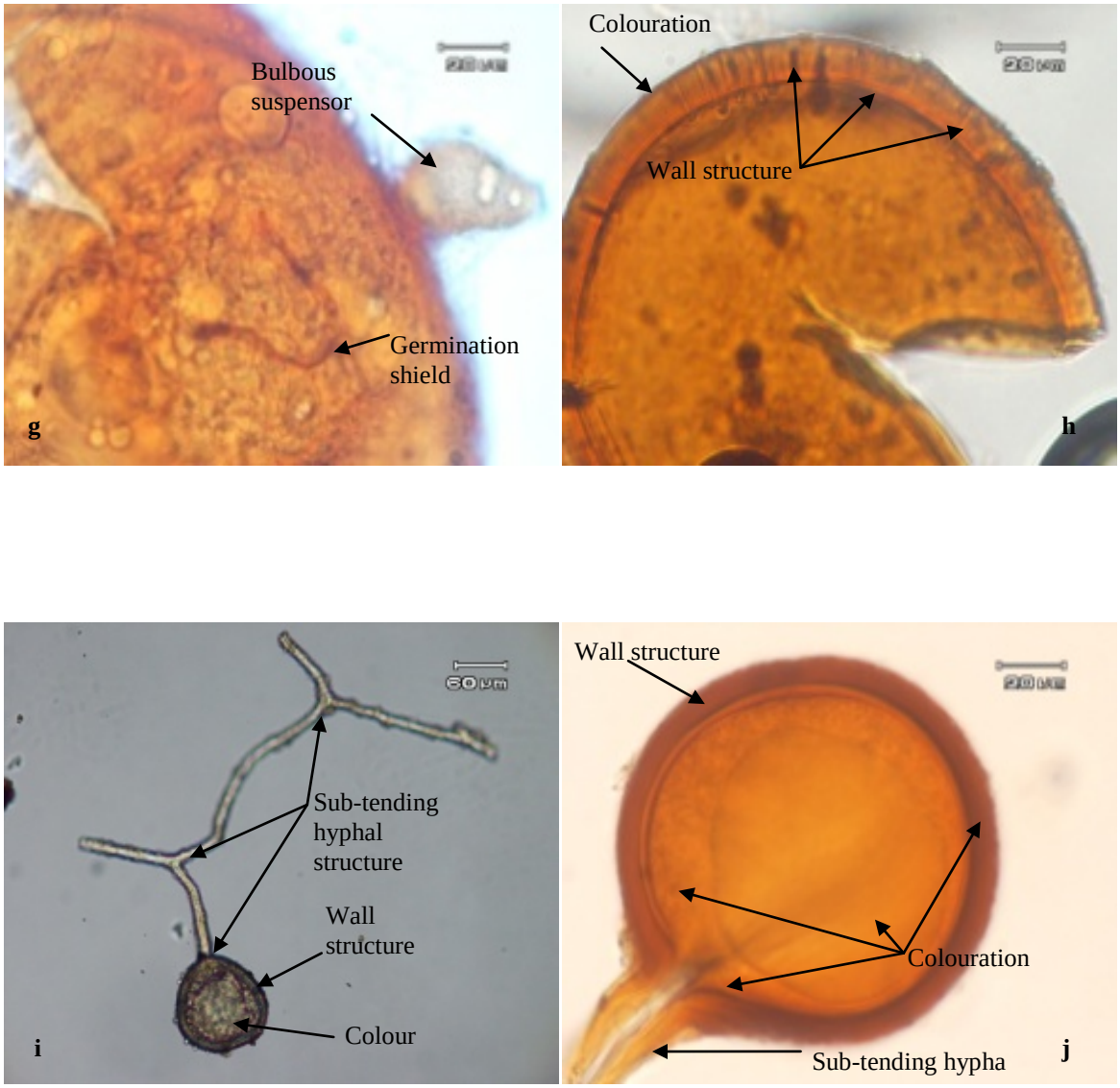


Plate 3.3. Micrographs of 10 of the 16 AM species spores extracted from transect soils describing major morphological features that substantiate robustness of identification, size the first criterion. **a)** *A. scrobiculata*, absence of sub-tending hypha, atypical ornamentation, and inner walls staining in Melzer's reagent **b)** *A. spinosa*, no sub-tending hypha, distinct spines ornamentation on outer wall, saccule remnants attached from which all *Acaulospora* spp. spores uniquely develop **c**) *A. dilatata*, distinctive ornamentation, yellow-brown colouration, and walls structure **d**) *Gi. margarita*, family trait bulbous suspensor, inner walls staining red in Melzer's reagent **e**) *S. gregaria*, family trait bulbous suspensor and germination shield **f**) *S. scutata*, family trait bulbous suspensor and germination shield, distinctive colouration **g**) *S. calospora*, family trait bulbous suspensor and unique germination shield **h**) *G. fasciculatum*, colouration and wall structure **i**) *G. albidum*, colour, wall structure and sub-tending hyphal structure **j**) *G. claroideum*, colouration, sub-tending hypha, walls structure.

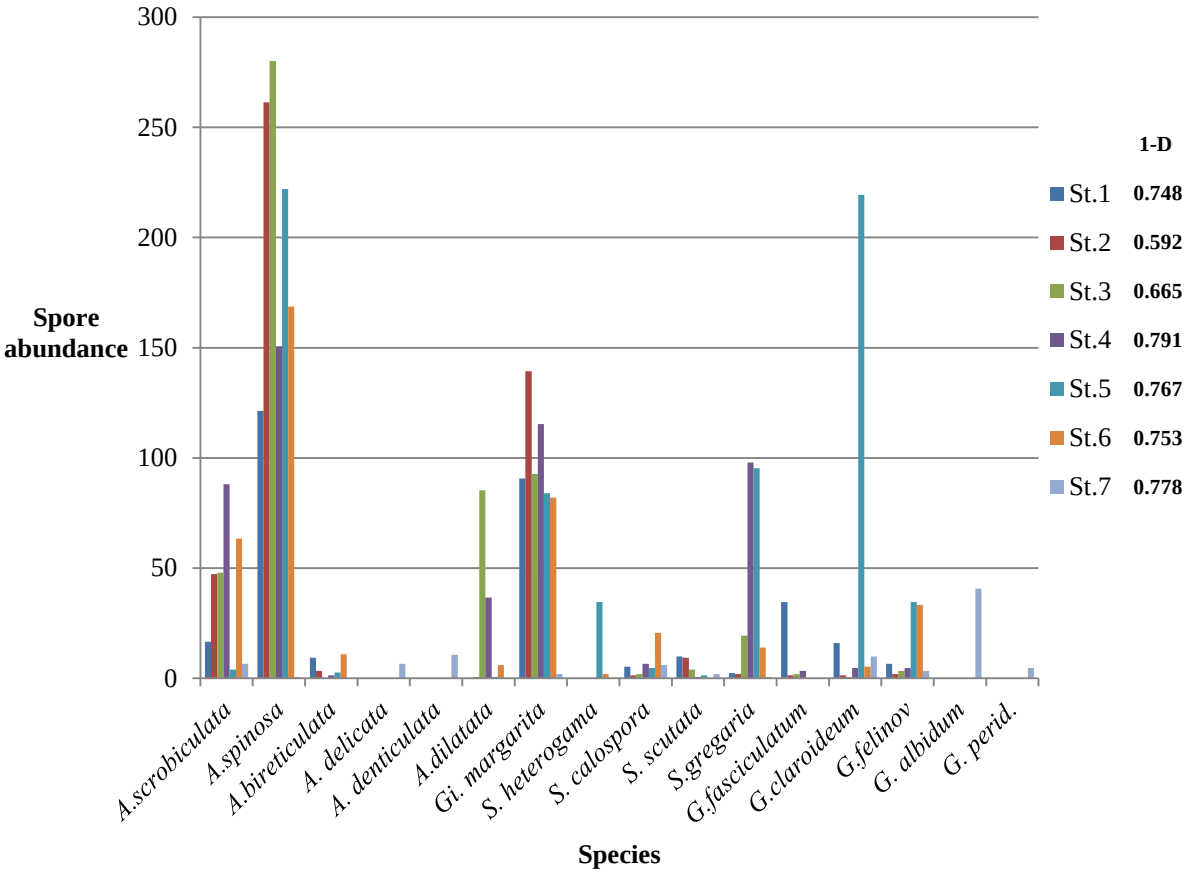


Fig. 3.12. Pre-monsoon spore abundance (25.5.12) at species level, showing the relative abundances at each station on the transect. *G. perid.* = unidentified peridial *Glomus* sp. 1-D is incorporated into histogram legend. For SD see **Fig. 3.14**.

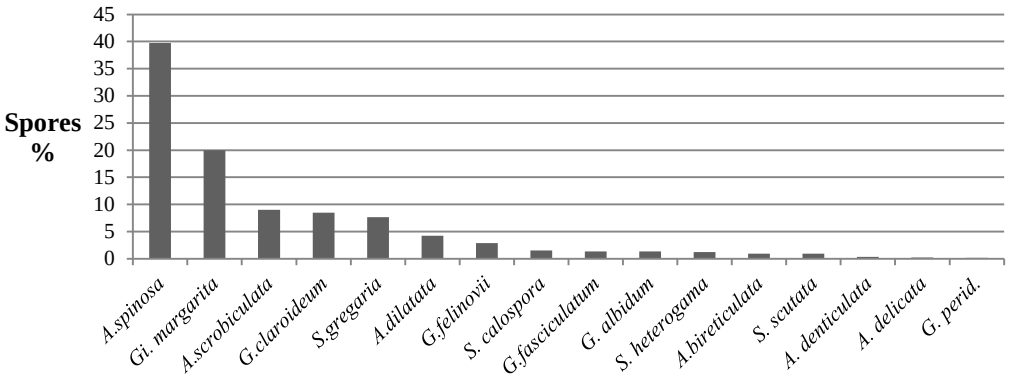


Fig. 3.13. Pre-monsoon (25.5.12) species abundance of AM fungal spores in Rank Order. (*G. perid.* = unidentified peridial *Glomus* species).

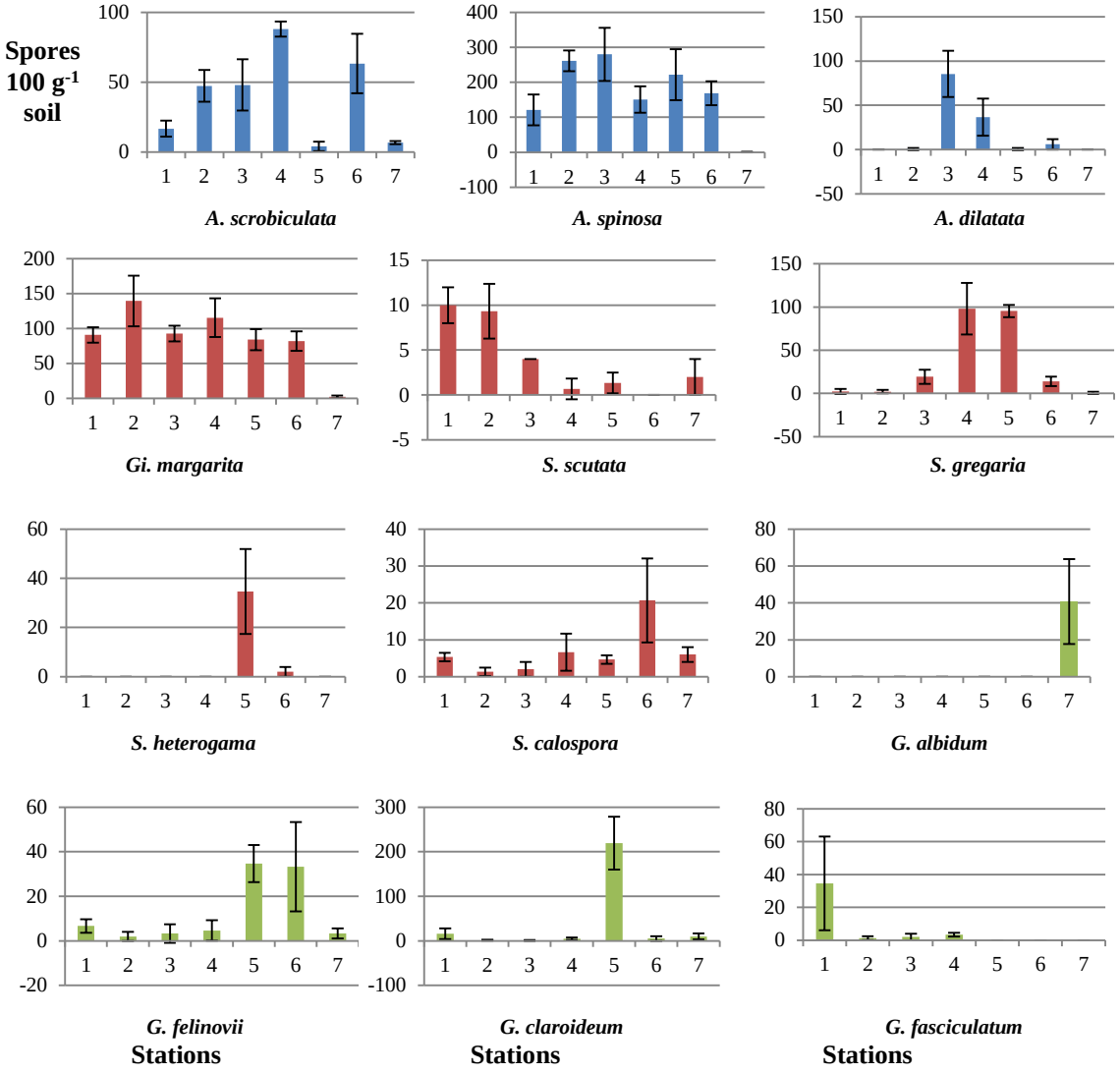


Fig. 3.14. AM fungal spore abundance of 12 of the 16 species (98.4% of total spores recovered) extracted from 100 g^{-1} sub-samples of rhizosphere soils extracted pre-monsoon (25.5.11) from 7 stations on a transect in a primary coastal sand-dune system on the west coast of India. n=3 x 50 g. Error bars represent SD.

Figure 3.15 is a CCA triplot of plant species, distance and AM spore abundance at species level (25.5.12) over the transect, showing the comparative distributions of plant and AM spore assemblage over distance (stations). Limited amplitude of arrows representing spores distribution strongly indicates plant species have no influence on AM species distribution, and *vice versa*.

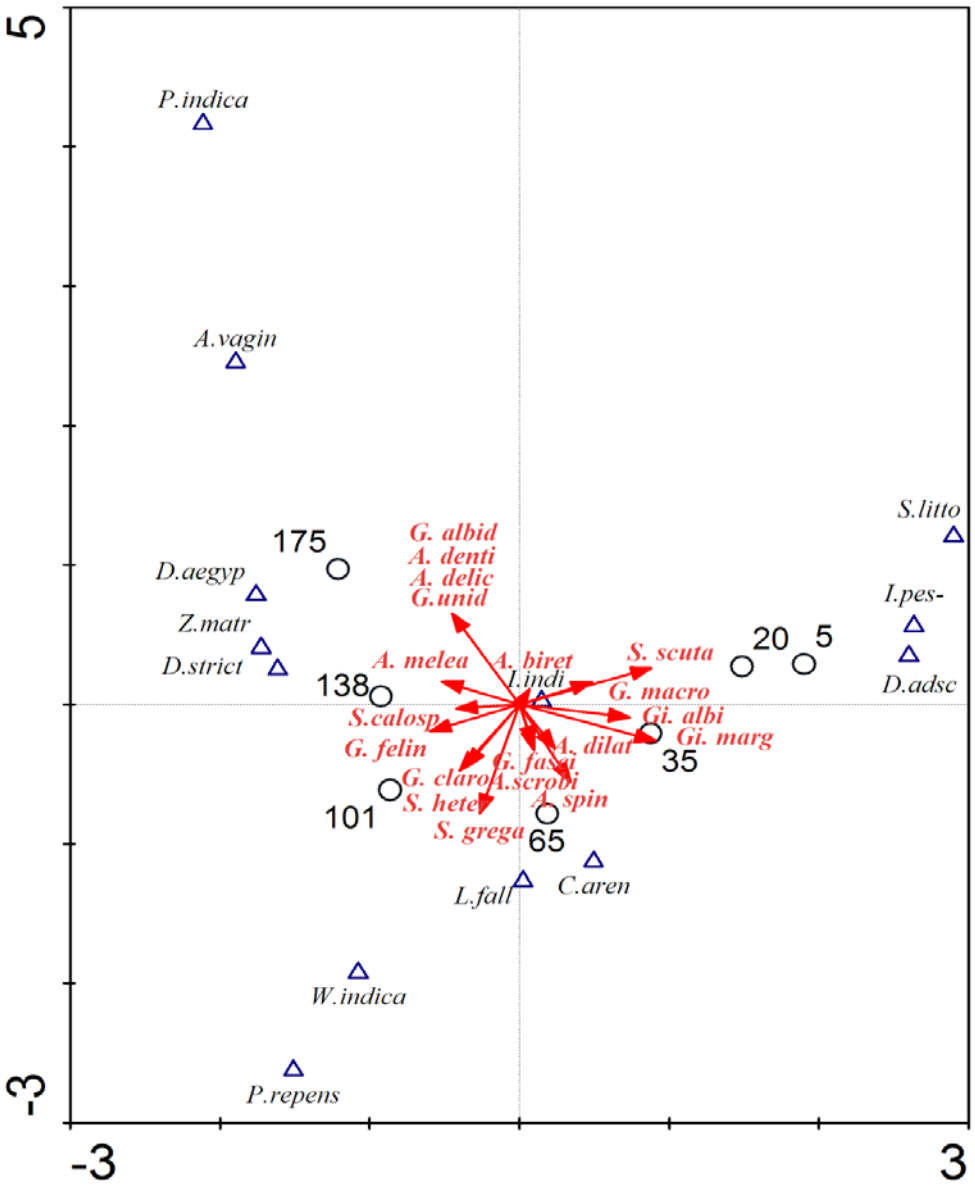


Fig. 3.15. CCA triplot showing ordination of plant species (triangles), station locations on the transect (circles) and pre-monsoon spore abundance at species level (arrows). For full list of plant species names see **Table 3.1**, and for spore names see **Fig. 3.12**. The two axes jointly explain 70.9% of the variation.

The CCA triplot of station locations, soil chemistry and spores abundance and diversity at species level (Fig. 3.16) indicates there may be association between particular AM species and certain soil chemistry factors. Correlation analysis (Table 3.4) shows there is strongly significant association of *Gi. margarita* with pH (r = 0.885; *P* = 0.008) and significant

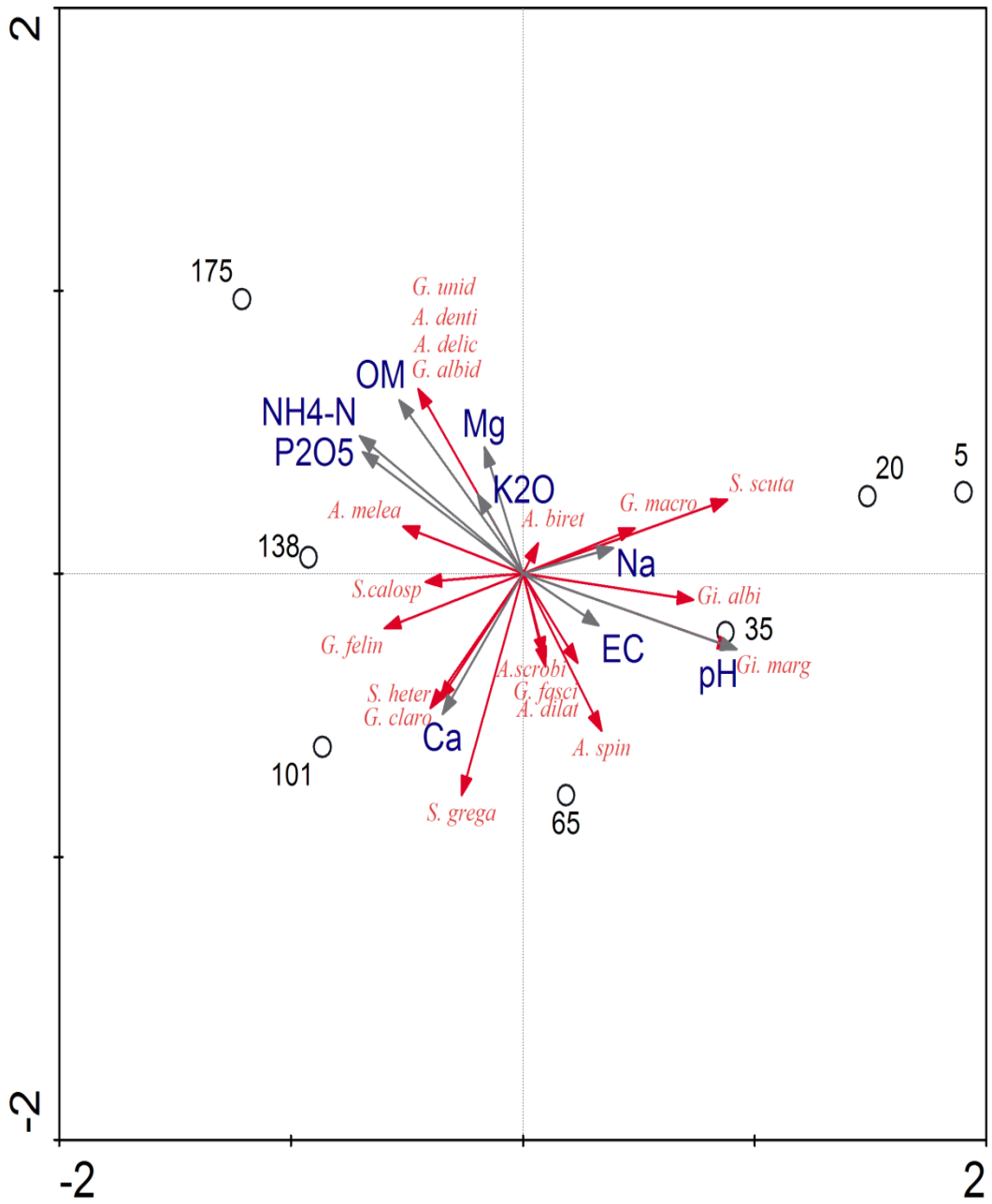


Fig. 3.16. CCA triplot showing ordination of station locations on the transect (circles), soil chemistry (grey arrows) and spores at species level (red arrows). For full list of spore names see **Fig. 3.12**. The two axes jointly explain 70.9% of the variation.

Table 3.4. Correlations of AM taxa and edaphic factors confirming associations indicated in CCA ordination **Fig. 3.16**.

	r	*P*
Gi. margarita : pH	0.885	0.008
G. felinovii : Na	-0.774	0.041
S. calospora : Na	-0.814	0.026
A. spinosa : OM	-0.819	0.024
A. spinosa : P_2O_5	-0.735	0.060
Gi. margarita : OM	-0.878	0.009
Gi.margarita : P_2O_5	-0.859	0.013

negative association of *G. felinovii* and *S. calospora* with Na (r = -0.774; *P* = 0.041: r = -0.814; *P* = 0.026 respectively). There is also significant negative association of *A. spinosa* with OM (r = -0.819; *P* = 0.024) but non-significance with P_2O_5 (r = -0.735; *P* = 0.060), and strong significant negative association of *Gi. margarita* with OM and P_2O_5 (r = -0.878; *P* = 0.009: r = -0.859; *P* = 0.013). The correlation matrix (WebAgris 2.0) indicated there was weak significant negative correlation (r = -0.782; *P* = 0.059) of Gigasporaceae with soil water content. A significant negative (r = -0.880; *P* = 0.024) relationship was indicated between *Glomus* and <52 µm sand-grain particle size and a significant positive (r = 0.895; *P* = 0.020) relationship between *Glomus* and 250-500 µm particle size.

Tukey's grouping of significant difference in Simpson's index of diversity (1-D) ANOVA over the entire transect, plants, spore abundance at species level pre-monsoon, and genus level pre- and post-monsoon (F = 9.56; *P* = <0.001), is presented in Table 3.5. There was no significant difference between the four categories in St. 1-3 but there was significant difference between the four in St. 4-7 (F = 20.97; *P* = <0.001). Tukey's grouping St. 4-7 is shown in Table 3.6.

Table 3.5. ANOVA of Simpson's index of diversity (1-D) of plants, AM spore abundance at genus level pre- (25.5.12) and post-(4.12.11) monsoon, and spore abundance post-monsoon at species level, St. 1-7.

	n	Mean	Grouping	
Spores 25.5.12 species level	7	0.698	A	
Plants	7	0.696	A	
Spores 25.5.12 genus level	7	0.538		B
Spores 4. 12 11 genus level	7	0.498		B

Means that do not share a letter are significantly different.

Table 3.6. ANOVA of Simpson's index of diversity of plants, AM spore abundance at genus level pre- (25.5.12) and post-(4.12.11) monsoon, and spore abundance post-monsoon at species level, St. 4-7.

	n	Mean	Grouping		
Spores 25.5.12 species level	4	0.772	A		
Plants	4	0.690	A	B	
Spores 25.5.12 genus level	4	0.575		B	C
Spores 4.12.11 genus level	4	0.478			C

Means that do not share a letter are significantly different.

3.5. Discussion

3.5.1. Plant community

Plant species diversity in stations was limited, typical (Ranwell 1972, Maun 2009) of a nutrient deficient, structurally simple psammite matrix primary dune-system. A total number of 13 plant species were recorded along the transect. Simpson's diversity was variable at low levels from station to station (Table 3.1) but overall was relatively high (1-D = 0.816) when compared with other plant communities, 0.599 in restored prairie grassland in N. America (Carter and Blair 2012), 0.880 across a range of grazed pasture in north-east Spain (de Bello, Lepš, and Sebastià 2006). CCA (Fig. 3.1) supports the visual impression of plant zonation along the transect indicated in Table 3.1. Data further indicates plant/plant facilitation, or at least non-competitiveness, where correlations between frequencies of *I. pes-caprae* and *D. adscendens* (r = 0.970; *P* = 0.003) in St. 1 to 4, *L. fallax* and *W. indica* (r = 0.905; *P* = 0.017) St. 4 to 6, and *D. aegyptium* with *Z. matrella* (r = 0.858; *P* = 0.031) St. 5 to 7 were positively significant.

Ipomoea pes-caprae and *S. littoreus* were observed to rapidly colonize the full 2-3 m width of newly-formed incipient berm at the foot of the established dune ridge during the hot, dry, and windy early months of the year (Plate 3.4), ramet roots helping stabilize sand-grain particles (Maun 2009). Ramet roots of both species were found to be colonized by AM fungi, Koske and Gemma (1990) suggesting re-established *I. pes-caprae* fragments often carry viable AM inoculum in roots after immersion in the sea.

Plate 3.4. Berm invasion by extensive fast-growing stolons of *I. pes-caprae* and *S. littoreus* from established genets higher up in the dune. Date: May 2011

3.5.2. Soil and environment

The only clear gradient across the system was pH (Fig. 3.2a, Fig. 3.3), the others that were anticipated, OM (Fig. 3.2c), salinity (Fig. 3.2f) and NH_4-N (Fig. 3.2d) showing no clear delineation. OM, and consequently NH_4-N, remained very low in concentration up to St. 7 where there was a sharp increase, deficient still (c.f. Willis and Yemm 1973, Lammerts and Grootjans 1997), but adequate to influence increase in plant frequency. Sodium was relatively evenly distributed along the transect, highest in the pre-rains series that equates with a similar observation in EC (Fig. 3.2b). The negative correlation of pH with OM conforms to common soil chemistry activity where microbial N-degredation activity increases acidity (Brady 1974, Yan, Schubert, and Mengel 1996), sharp increase in OM/NH_4-N concentration coincident

with similarly sharp increase in soil acidity at Station 7. There is a negative correlation of pH with P_2O_5 on the spatial scale that actually belies P_2O_5 variation with time (Fig. 3.2e), on occasions deficient, as low as 4 µg g^{-1}, at other times attaining plant nutrient-sufficiency levels of 40 µg g^{-1} (Bagyaraj and Balakrishna 2003) and above, even within the same time series. Interestingly CCA (Fig. 3.4) indicates positive association of OM with P_2O_5 which suggests plant available P may be complexed in OM. There are also strong positive correlations of OM and P_2O_5 with silt/clay content (Table 3.4). It cannot be ascertained what proportion of silt/clay might be complexed with OM but the data may suggest AM fungi scavenge PO_4 principally from OM and silt/clay. Phosphates released from Ca (Fig. 3.2h) and iron (Fe) in clay and loam soils can be substantial (Pierre and Parker 1927, Brady 1974) but may be negligible at the pH levels in this psammite matrix. There is no significant correlation of Ca with any factor other than a weak negative association with Mg (Fig. 3.2i). CCA (Fig. 3.4) and subsequent correlation indicates association of Mg with OM but stronger correlation with silt/clay (Table 3.4). This suggests the mineral resource may be complexed more with silt/clay. Canonical correspondence analysis shows association of Mg with K_2O (Fig. 3.2g) that again is correlated significantly more with silt/clay than with OM (Table 3.4) indicating K^+ is gained more from silt/clay. The low OM levels recorded up to Station 7 are similar to results found in south India by Karthikeyan and Selvaraj (2009), 0.12-0.67% (range 0.18-0.34% in this study), as are P_2O_5 levels, 15.5-53.6 µg g^{-1} (range 4.2-50.0 µg g^{-1} in this study), but Ca lower than the levels found in this study site.

Fluctuation about means in Ca, Mg and K_2O data may suggest a dynamic interchange in exchangeable bases, intensified by variable high rainfall, percolation and leachate rates and the pH gradient across the transect. Concentrations of Na (Fig. 3.2f), the least soil colloid-adsorbed ion (Brady 1974) were relatively low during monsoon, oddly lower still in analyses

undertaken during the dry season (Fig. 3.9), and the weak correlation with EC suggests there is little saline-induced stress effect within the system. Sodium, often equated with EC, is described as a major limiting factor in plant growth in coastal dune systems (Wilson and Sykes 1999) and indeed a general halophyte adaptation to high salinity is demonstrated by reduced biomass production and plants remaining prostrate. Levels at which NaCl become toxic vary with plant species, Sahrawat *et al.* (2009) finding salt-sensitive pigeonpea (*Cajanus cajan* (L.) Millsp) adversely affected at EC 0.83 dS m^{-1}, and detrimental effects are often related to osmotic potential reversal affecting water relations, particularly in germination and development at seedling stage (Rozema *et al.* 1985). Sodium has also been shown to effect reduction in plant available ammonium in some soils (Wali, Kumar, and Singh 2003, Green, Machin, and Cresser 2008). Results here, however, seem to be at variance with documented evidence. Sodium concentration in coastal dunes in some reports is similar to that recorded here, e.g. 92 µg g^{-1} in rhizosphere soils of *Distichlis spicata* (L.) Greene on a California dune system (Allen and Cunningham 1983) but there are reports of higher concentrations (e.g. >300 µg g^{-1}, Mariko *et al.* 1992). Electrical conductivity that on no occasion attained equivalent levels of Na associated toxicity described in saline sensitive pigeonpea referred to above, remained comparatively very low during the last two time series throughout the transect line, possibly as a consequence of leaching. However, Na concentration remained reasonably constant throughout the time series over the transect, oddly rising slightly towards Station 7, the furthest from the sea. This suggests water-soluble salts other than Na were leached. Magnesium was the only mineral to resemble the EC pattern of late series decline but with weak correlation, and the remaining Ca, P_2O_5 and K_2O all retained near-initial levels.

Analysis of soil chemistry data at the temporal scale describes difference in the transect as a whole, over time. The analysis indicates the level of stability and evenness of soil chemistry characteristics and nutrient availability through the growing season. EC is strongly correlated with Na (Table 3.3) but ANOVA suggests the temporal patterns of change differ. Pre-monsoon (Series 1) EC is significantly greater than post-monsoon (Series 5), pre-monsoon significantly greater than Series 2 & 3 and Series 3 & 4 significantly greater than Series 5, where Na is significantly greater only in Series 1 (Table 3.2) and Series 2-5 relatively stable. Change in EC also correlates significantly with pH (Table 3.3), and there is no significant ANOVA difference, suggesting changes over time are relative to each other. Correlation of EC with K_2O is non-significant, and there is no significant ANOVA difference, suggesting K salts have little effect on EC. EC is further correlated with OM, here negatively, but with no significant ANOVA difference. Interpretation of this statistic is difficult to comprehend where there seems to be little difference in the OM series over distance, St. 1-6 ranging from *ca* 0.02% - 0.05%, and 2-way ANOVA indicates distance is the primary influence (Fig. 3.2). Either EC is highly variable and OM is not, or OM, even though in such small concentration, is also highly variable over time. The time-scale used, perhaps too coarse, has not indicated where the variability may lie. K_2O is correlated with pH (Table 3.3), again ANOVA indicating non-significant difference, suggesting increasing acidity may be affecting K nutrient availability. K_2O, OM and NH_4-N are grouped together in ANOVA, the latter two as might be expected, N having derived from OM. However, K_2O : NH_4-N correlation is significantly positive whereas K_2O : OM correlation is significantly negative (Table 3.3). The statistic is perplexing, and not open to clear interpretation, except perhaps that NH_4-N data were gained from transect region St. 4-7 only, which appears to be partitioned from St. 1-3. There is strongly significant negative correlation between Ca and Mg (Table 3.3), in an ANOVA grouping significantly different from all other nutrients, Mg Series 1, 2 & 3

significantly greater than Series 4 & 5, interestingly reversed in Ca, Series 4 and 5 significantly greater than Series 1, 2 and 3. Again the phenomenon cannot be explained. The OM : Na correlation has been included in Table 3.3 even though it is not significant as each is placed in a significantly different group in ANOVA Tukey's grouping, suggesting Na may not be complexed in OM.

Levels of soil water content 2 h and 4 d after rain (Fig. 3.6) showed strongly similar pattern over the transect, each rising in St 7. Correlation with OM was significant in both instances, strongly so in the 4 d data, indicating all retained moisture was held in OM after percolation. Negative correlation of soil water content with 52 µm sand-grain particle size (Fig. 3.7) and positive correlation with 250-500 µm particle size seems anomolous where percolation through the larger size might have been anticipated to be the greater. It may be explained by water-retentive organic materials being involved in sand grain aggregation (Six *et al*. 2004)

3.5.3. Spore community

Taxonomic diversity of AM fungi was limited when compared with studies made by Picone (2000) in tropical grassland where 28 distinct fungus morphospecies were described, with Husband *et al*. (2002) where a total of 30 AM fungal types were identified in tropical forest in Panama, with Zhao, Wang, and Yang (2003) who identified 27 species of AM fungi in tropical rainforest in southwestern China, and with Öpik *et al*. (2008) who recorded 34 Glomeromycota taxa in boreal herb-rich coniferous forest. Here only 16 AM species were recovered, similar to 12 species reported by Cordoba *et al*. (2001) in Brazilian dunes and 14 species isolated from Hawaiian dunes by Koske and Gemma (1996).

3.5.3.1. Genus level

There was variation in spore abundance between post- and pre-monsoon data at genus level, although there are no ANOVA significant differences in pairwise datasets, where all three genera increased in abundance. This may support the suggestion of dessication and decrease in mycelium density during the hot, dry months encouraging sporulation. Principal components analysis (Fig 3.11) indicates there is no consistent association of AM spore distribution with plant community zonation. Hart and Reader (2004) suggested that Gigasporineae (Gigasporaceae) inoculate primarily by spore, an advantageous strategy in this environment, and Glominieae (*Glomus*), here in low spore-number, inoculate primarily by hyphae. Lekberg *et al*. (2007) further suggested AM fungi in the family Glomeraceae disperse more efficiently in clay soils, whereas AM fungi in the family Gigasporaceae disperse more readily in sandy soils. These observations may explain some of the reason for low recovery of *Glomus* spores across the transect. The demographic shifts in transect positions of peak abundance cannot be explained where spore numbers of *Acaulospora* species remained at approximately the same level in St. 3 pre- and post-monsoon but in St. 5 reduced by >65%, and Gigasporaceae peak abundance, at increased levels, shifted from St. 3 to St. 4. Two-way ANOVA showed no significant influence of time or distance on either spore abundance or on Simpson's diversity (Fig 3.10a, b).

Correlation between partial St. 1-3 and St. 4-7 transect OM and spore abundance however, proved strongly significant, positive relationships between OM and all AM fungal taxa spores in the parting St. 1-3, but negative correlation with *Acaulospora*, and non-significant negative correlation with Gigasporaceae and *Glomus* in St. 4-7. A similar partitioning is seen in OM : pH correlation, weakly negative in the part St. 1-3 and significantly negative in the part St. 4-7. These changes coincide with mean decline from pH 6.6 in St. 3 to pH 6.5 at St.

4, the 65 m sheltered area behind the foredunes, suggesting that region on the transect may be a transition zone. The data indicate no clear explanation of this but the literature suggests pH can have a direct effect upon soil OM, You, Yin, and Allen (1999) showing increase in pH at this range causes increase in dissolution of soil organic matter (SOM), and Curtin, Campbell, and Jalil (1997) described that when pH of two slightly acid (pH 5.7 and 5.8) soils was raised to pH 7.3 and 7.4 using $Ca(OH)_2$, mineralization of N and C was stimulated, attributing release of labile OM to be a direct response to the pH increase. Should this be happening in the foredunes (St. 1-3), plant dependency on AM association would be lessened where an available labile nutrient resource can be taken up *via* the direct pathway (see Glossary) in addition to, and in excess of, nutrient transported *via* the indirect mycorrhizal pathway.

3.5.3.2. Species level

Relative abundance in species rank order (Fig. 3.13) indicated mycobionts followed the universal hollow curve of organism abundance, fewer common than rare species (Darwin 1859, McGill *et al*. 2007). *Acaulospora spinosa* was at high abundance in every station but the last, *Gi. margarita* and *A. scrobiculata* similarly represented but at reduced abundance. The majority (81.4%) of *S. gregaria* spores were recovered from St. 4 and 5. All other species were at far reduced abundance, and variable over distance. A strong positive correlation ($r = 0.984$; $P = 0.001$) was found between *G. fasciculatum* (Thaxter) Gerd. & Trappe emend Walker & Koske and *I. pes-caprae* but care should be taken in interpreting this as specificity or even preference without further investigation. Beena *et al*. (2000) described *G. fasciculatum* spores extracted from rhizosphere soils of five plant species other than *I. pes-caprae* in dunes on the coast of S.W. India, none of which were recorded in this survey. Selvaraj and Kim (2004), in a survey of three dune systems on the tip of India, recorded only

five (of 16) AM fungal species common with this Goa study, the dominant species *A. spinosa* entirely absent. Jaiswal and Rodrigues (2001) reported *A. spinosa* and *Gi. coralloidea* Trappe, Gerd. & I. Ho the most frequently occurring AM species spores in a survey of nine selected plant species on Colva Beach dune system in south Goa, only one of which was common to this study site. They also reported recovery of an AM species from genus *Sclerocystis* Berk. & Broome where none were recorded at the Morjim site. Such evidence suggests AM, and plant, community structure may vary considerably from one site to another.

Principal components analysis (Fig 3.11) had indicated there may be association of post-monsoon AM genera with plant community zonation. However, over the entire transect there was no association of AM fungal spore taxa abundance or diversity recorded at species level in the post-monsoon dataset with plant demography. Limited length of arrows in CCA (Fig. 3.15) indicates little association of AM spore abundance with plant species distribution and suggests the two community compositions are independent of each other. This evidence supports the hypothesis of non-specificity in AM fungi where all (excepting *C. arenareus*) plants were mycorrhizal, and dependent upon the symbiotic association characteristic of the extreme environment.

The comparative high-abundance recovery in St. 5 and 6 (Fig. 3.12) of *Glomus* spp. *G. claroideum* and *G. felinovii* sp. nov. (proposed: GenBank ID BankIt 1599364 Seq. KC603764 – see Appendix 1.3) is anomalous and there is little evidence to indicate why this may be so. CCA (Fig. 3.16) shows *G. felinovii* lies on an axis opposite to Na and correlation was found to be signicantly negative ($r = -0.774$; $P = 0.041$). The analysis also shows *G. claroideum* to be associated with Ca but here there is no significant correlation. Topographical variation may have had an effect, the soil surface at St. 5 fluctuating at a *ca* 200-300 mm higher level

than the previous station (Fig.2.2) and consequently soil-water would percolate through a greater depth than in previous and following stations (Fig. 3.6). If that is the case then mycelium may have been particularly prone to desiccation and a strategy of high sporulation rate in an area occupied by *Acaulospora* and Gigasporaceae in declining abundance (Fig. 3.10) and unoccupied by competing *Glomus* spp. (Fig. 3.12) would gain considerable advantage.

The CCA triplot of distance, soil chemistry and species-level spore abundance (Fig. 3.16) indicates further associations of particular species and certain edaphic factors. Figure 3.17 shows a number of significant correlations of AM taxa other than that of *G. felinovii* : Na described above. The only positive correlation strongly suggests the more acidic the soil, the fewer the *Gi. margarita* spores but the statistic may be suspect. Had the spore abundance in St. 4 been 23% less than that recorded the argument might have been much stronger. There may also be adverse influence where distribution is heavily skewed to the right as at St. 7 there is almost half a magnitude increase in acidity over the previous station and no AM species *Gi. margarita* detection. Nevertheless there is strong association between the two. Negative correlation is less open to interpretation. There is slight decrease in Na means (Fig.3.2) that corresponds with *G. felinovii* abundance in St. 5 and 6 supporting the above argument, and similar correspondence in *S. calospora*. Association and significant correlation of *A. spinosa* with OM and non-significant association with P_2O_5 suggests the taxon may be preferentially scavenging nutrient from the former but this argument breaks down in interpretation of significant negative correlation of *Gi. margarita* with both OM and P_2O_5. Comparative station by station data of silt/clay content that is shown to be a further source of P_2O_5 (Table 3.5) is not available. What the data examination does suggest is that the AM fungal community is driven by soil chemistry factors.

Biotic and abiotic soil characteristics changed abruptly at St. 7. Soil acidity increased by 13% over the previous station, OM by almost 350%, NH_4-N by >380%. Although percentage increases are large, actual increases were small. Nevertheless, there was notable effect. Plant frequency of *I. indicum* was 100%, an increase of 33% over the previous station. Soil silt/clay percentage content increased 12-fold over St. 6 and soil water-capacity retention was >205% higher. Sand-particle size (<52 µm : 250-500) ratio increased by 13.5%. Soil colour was a light reddish-brown. It is proposed that this region of the transect is a transition zone in succession, a solum-ecotone, from psammite to humic soil. It might also be described as an AM fungal ecotone. *Acaulospora* and Gigasporaceae species spore numbers that had been declining in the previous two stations fell to comparatively negligible levels and *Glomus* species that includes the dominant *G. albidum* not recovered on the transect previously, were the most abundant.

3.6. Conclusions

- The evidence indicates there is a distinct zonation pattern in plant community along the transect.
- Soil chemistry has proved variable both spatially and temporally, despite a simple psammite structure. A pH gradient towards acidity is indicated at the spatial scale. There is transect-wide variation in all investigated factors at the temporal scale.
- Plant nutrients are derived from both OM complex and silt/clay content.
- Plant zonation does not correlate with soil chemistry analysis.
- Plants assemblage and AM spores assemblage bear no relationship with each other.
- Spores assemblage is driven by soil nutrients chemistry.
- Spore abundance data shows *A. spinosa* and *Gi. margarita* to be co-dominant AM taxa in all stations but the last where *Glomus* spp., albeit at low density, are dominant.
- St. 7 is a pedological transition zone that coincides with the marked change in AM spore abundance and diversity.
- pH : OM and spore abundance : OM correlations, and Simpson's ANOVA, suggest there are distinct partitions in the transect, St. 1-3 and St. 4-7.
- There is indication that certain AM taxa may be functionally associated with particular soil nutrients, but no evidence that there is nutrient-function efficiency.

CHAPTER 4

A PRELIMINARY FIELD TEST OF AM FUNGAL FUNCTIONALITY: SPORE DIVERSITY AND DENSITY IN RELATION TO NUTRIENT AMENDMENT IN TWO CONTRASTING STOLONIFEROUS PERENNIALS IN THE FOREDUNES

Knowledge is an unending adventure at the edge of uncertainty.
Jacob Bronowski

Plate 4.1. Ramets (see Glossary) of the studied stoloniferous clonal plant spp., in pots inserted into foredunes. **a**) *Ipomoea pes-caprae* L. (Convolvulaceae), **b**) *Spinifex littoreus* L. (Poaceae).

4.1. Introduction

A trial early in 2009 had been undertaken in a replicated (n = 3) 25 µm nylon membrane (preventing root but not AM hyphal access) split-pot. The pots contained dune mesocosms transferred from St. 3, 5 and 7 regions in the Chapter 3 transect in one compartment, and sterilized dune sand with rock-phosphate (RP) amendment in the other (Plate 4.2). Arbuscular mycorrhizal (AM) spore abundance and diversity analysis showed a high abundance of *Acaulospora dilatata* Morton in all three amended compartments after 36 weeks. It was

postulated, and subsequently hypothesized, there is taxon specific nutrient-function efficiency in AM fungi.

Plate 4.2. Mesocosms of transect plants community transferred to split-pots, rock-phosphate amended sterilized soils in the covered compartments.

Sequestration, transport and contribution of nutrients to host plants is a major functional feature, and the most investigated, of the AM fungal symbiosis (Smith and Read 2008). The function varies inter-generically and inter-specifically (and maybe even intra-specifically) in the volume and flux of nutrients acquired and delivered (Smith, Smith, and Jakobsen 2003), and perhaps also, it was considered, in efficiency of delivery of any one particular nutrient. Species *X* may be more efficient in the contribution of phosphates (P) and species *Y* more efficient in say zinc (Zn), or ammonium nitrate (NH_4-N), to the host, and such specificity may be strategically advantageous to a fungal species. All AM fungal species compete for the same resource, photosynthetically derived carbon (C), and should any taxon be more efficient in the sequestration and transport of a particular nutrient the host is in need of, reciprocal C

exchange on the part of the host may be preferential. Recent evidence (Kiers *et al*. 2011, Smith and Smith 2012) clearly shows both partners in the symbiosis have an element of control over the exchange mechanism.

Chapter 3 had indicated that in the hostile environment of the tropical primary coastal sand dunes almost all plant species are mycorrhizic and that certain AM taxa may be associated with particular soil nutrients. Coastal sand dunes are noted for heterogeneity of deficient nutrient resource, a distribution at a three-dimensional meso- and even micro-scale (Caldwell, Manwaring, and Durham 1996) that is as patchy as are plants. The limiting factor to plant growth over the whole system might be shown to be one particular resource, often nitrogen (N) (Olff, Huisman, and van Tooren 1993), but should a plants roots be in the vicinity of a patch of N resource, then immobile P, for example, that is soon subjected to nutrient depletion zone exclusion in direct-pathway uptake (Hinsinger 2001), may be that plants principal limiting factor. In the direct trade-off in the symbiosis (C for nutrients), the novel concept of a functionally-efficient AM fungal species amongst multi-species colonization delivering that limiting nutrient, and thus gaining an advantage that may lead to greater sporulation and thus fitness, has merit. Fungal specificity in a particular nutrient exchange may afford a competitive edge.

There is indirect support for the hypothesis in the literature. Avio *et al.* (2006) suggested that the efficiency of hyphal P uptake and transport is a species-specific fungal variable after having studied extraradical hyphal network variation in geographically disparate isolates of two *Glomus* spp. in *Medicago sativa* L. rhizospheres. Munkvold *et al*. (2004) had previously demonstrated, in a similar experiment, fungal efficiency variability in 24 different isolates across four *Glomus* spp. correlated with extraradical hyphal biomass. Smith, Smith, and Jakobsen (2004), on the other hand, found *G. intraradices* P supply greater in three plant

species where *G. caledonium* extraradical hyphal length was much higher. One of the parameters Jansa, Mozafar, and Frossard (2005) researched was the efficiency of uptake of soil P by AMF in their study of diversity in phosphorus acquisition strategies among eight isolates of arbuscular mycorrhizal fungi (AMF) belonging to three *Glomus* species, all obtained from the same field site. They suggested their findings indicated remarkable functional diversity in the underground component of the field site. Jansa, Smith, and Smith (2008) referred to competition among AM species posing a major challenge in interpreting experiments with mixed inoculations in their P uptake comparisons experiment. Fitter (2006), in his previous review, suggested multiple colonization might occur frequently if the recognition systems for colonization were generic, and if fungal success within the root varied with ability to supply phosphate to the plant. It is suggested all of this evidence of, and reference to, P uptake might equally well describe species nutrient-function efficiency. As might comparative AM fungi trials in agricultural and horticultural plants seeking consistent indication of increase in crop plants quantitative and qualitative parameters with particular species. Positive results could indicate bio-fertilizer efficacy of the fungal species, but may also imply AM fungal nutrient-function efficiency.

The concept explored in a field setting is more complex than it first appears however. Plant nutrient-use efficiency adaptive strategies in species from nutrient-poor habitats, e.g. a root allocation pattern directed towards the acquisition of nutrients that diffuse slowly to the roots, traits that lead to high nutrient retention, and high nutrient resorption efficiency (Aerts and Chapin 2000), may negatively affect MIP. Funk (2013), in her review on traits of invasive plants (although a rare event in primary dune systems, but illustrating plant plasticity in a hostile environment) associated with resource acquisition and resource conservation in low-resource environments, reported the majority of studies of nutrient-use efficiency in invasive

species having higher values than co-occurring native species. An obvious adaptive trait in this dune system is that all grasses are C_4 (Table 3.1) and the C_4 pathway confers, although with increased energetic costs, greater photosynthetic water use and nitrogen use efficiency (Brown 1978).

Complexity is further heightened by plant nutrient requirements being variable, between and within plant taxa. Broadley *et al*. (2003, 2004) described variation in shoot calcium (Ca) content across angiosperm orders, and variation in shoot P and organic-N concentrations as a species level trait. Temporal requirement may also be variable, such as increase in P demand at the time of seed production for example (Miller 2000), although much of this contribution may arise from previous uptake accumulated in plant tissue rather than directly from soil resource (Harper 1977). Nevertheless, the precept holds true, and was considered worthy of investigation.

Although survey of the dune system (Ch. 3) had unveiled a complex association between soil chemistry, plants and AM fungi, it was considered relative simplicity in the component parts, i.e. a simple low-nutrient psammite soil (Fig. 3.2, Fig. 3.7), and low diversity in both plants (Table 3.1) and AM fungal spore abundance (Fig. 3.12) shown in the St. 1 and 2 survey could reduce environmental noise (see Glossary) that, in a later sere stage for example, where interactions between abiotic and biotic components are far more complex (Sykes and Wilson 1991), might further complicate, even obscure, the definitive line of enquiry (Eberhardt and Thomas 1991).

To test the hypothesis, three nutrient amendments, with non-amendment controls, were introduced into rhizosphere soils in the foredunes region of the transect soon after the onset of monsoon. The survey had shown two of the three co-dominant plant species (Table 3.1), *Ipomoea pes-caprae* L. and *Spinifex littoreus* L. (Plate 4.1a, b) were confined there. Both are

clonal species, genets annually (pre-monsoon) producing multiple stolons with numerous ramets that root freely on the arrival of rains. There may be significant difference in AM spore assemblage between the two. Mean pH (Fig. 3.2a) had fluctuated around neutral (range 6.9-7.4), mean OM (Fig 3.2c) had remained consistently low at 0.29% (range 0.26-0.55), and P_2O_5 (Fig. 3.2e) had ranged widely, from 4-36 µg g^{-1}. Sand-grain composition was found to be over 96% of <52 µm size. Just three AM species (Fig 3.12) had shared co-dominance, 86.5% of total spores extracted, and in high abundance, *Gi. margarita* in St. 2 the highest recorded of the species over the whole transect.

The three nutrient amendments made were P in the form of RP, OM as dry and mineralized tree leaf-litter, and insoluble, immobile metallic zinc. Mean available P_2O_5 levels had been shown to be deficient in St. 1 and 2 in the Ch. 3 survey, 15.0 – 16.0 µg g^{-1} (Fig. 3.2e), and perhaps much of the reason for high spore abundance. Concensus of evidence clearly shows correlation between high levels of AM in host roots and rhizospheres and low soil P concentrations (Hayman and Mosse 1972, Mosse 1973, Bolan 1991) and there are many reports in the literature describing the positive effects of rock phosphate amendment to rhizosphere on AM fungal and plant growth, particularly dual-inoculation with phosphate solubilizing bacteria (PSB) (Barea 1991, Toro, Azcon, and Barea 1997). Godhino (2007) had indicated considerable abundance of PSB organisms on *I. pes-caprae* and *S. littoreus* root surfaces and in rhizosphere soils in Goa foredunes. It was anticipated P amendment to direct-pathway adequate plant-growth concentration might, as has been often reported (Hayman, Johnson, and Ruddlesdin 1975, Pairunan, Robson, and Abbott 1980, Marschner and Dell 1994, Miranda and Harris 1994), depress that effect and show variation in AM spore abundance compared with controls.

Mean OM was also low in concentration in St.1 and 2 (Fig. 3.2c), and again where that is the case, AM fungi flourish (Koske and Halvorson 1981, Karthikeyan and Selvaraj 2009).

Contrary to P amendment, however, some organic amendments have been shown to encourage the proliferation of AM fungi (St. John, Coleman, and Reid 1983, Joner and Jakobsen 1995) but only where roots and mycelia are not in common contact with the source (Hodge 2003). Leaf-litter was a convenient organic amendment readily collected from the Goa University grounds. The material was decalcified, brown, dry and brittle, the leaves skeletal. Care was taken to remove it from an area where plant roots had not penetrated as previously similar leaf-litter collected from the University grounds had been found to be invaded by AM colonized plant roots (Plate 4.3a, b). The litter was derived almost exclusively from *Ficus benghalensis* L. leaves.

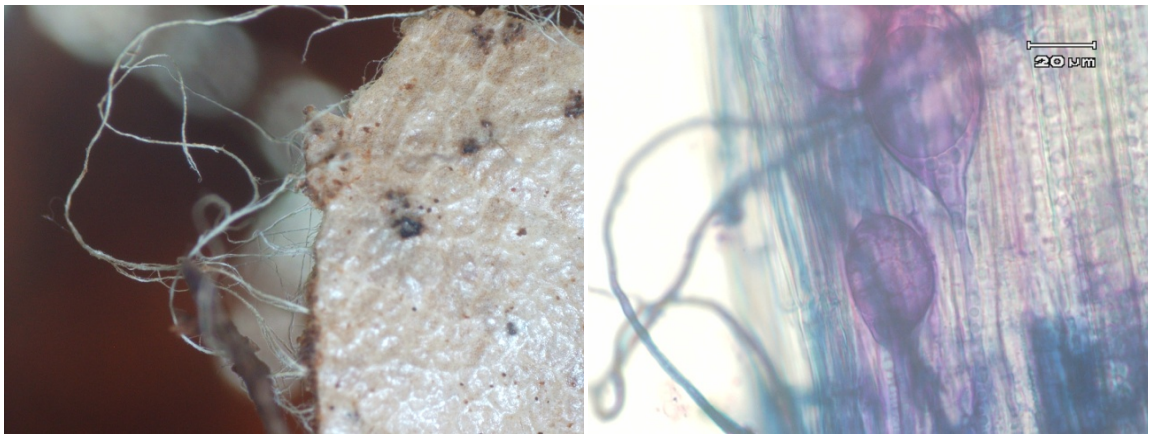


Plate 4.3. a) Roots invading leaf litter collected from Goa University grounds and **b**) stained AM vesicles in the invading roots. The litter proved an effective trap-culture inoculum, which may have considerable implication in the dissemination of AM propagules.

The tree leaf-litter was considered a relatively short-term nutrient resource when compared with the accumulated OM in the foredunes that, as Maun (2009: pp 32) has described, takes a greater length of time for decay. Nevertheless, even at the biochemical lower limits of P and N concentrations in senesced leaves reported by Aerts and Chapin (2000), about 3 mg g^{-1} N and 0.07 mg g^{-1} P, nutrient contribution, particularly low-rate leached N, P and Ca (Swift, Heal, and Anderson 1979), during a single rainy season could be significant.

The third amendment, metallic Zn, is highly immobile in soil, and AM fungi have been reported to increase Zn absorption, and accumulation in roots, at all addition levels (Chen *et al*. 2003). Bürkert and Robson (1994) described variable Zn uptake in three fungal species. The element is essential in carbohydrate transformation, participates in chlorophyll formation, prevents chlorophyll destruction (Salisbury and Ross 1978), and is essential for the function of many enzymes (Vallee 1976). It is an integral part in synthesis of the growth hormone indole-acetic acid (IAA) (Broadley *et al*. 2007) that, it was considered, is perhaps required in larger quantities during extensive stolon development exhibited by the two clonal plant species. Arbuscular mycorrhizal response to the amendment may be reflected in increase or decrease in spore abundance, depending on whether function enhances or inhibits uptake. Neutral pH in foredunes, where Zn concentration in the labile soil pool is known to increase as pH decreases (Reichman 2002), perhaps excludes the inhibition variable.

Spore abundance had proven a valuable parameter in the Ch. 3 survey and was again used here. It was anticipated that, in the relatively homogenous dune-crest habitat, any consistency in variance of spore species abundance or diversity that aligns with amendment treatments, or species isolated that were not present in the Ch. 3 survey, may indicate AM species-efficiency in nutrient acquisition. Also evidence that may arise indicating a particular host plant is driving AM fungal demography (Smith *et al.* 2010) may indicate preference in association. The 'field' approach is novel however, complicated, inherently complex, and the outcome is uncertain. Thus the study was a preliminary examination of the applicability of experimental parameters and design as much as it was a test of the hypothesis.

4.2. Objectives

- To determine the effect of three soil nutrients amendments, P as rock-phosphate (RP), metallic-Zn and OM leaf-litter, added to two disparate foredune clonal plant species ramets rhizosphere soils, on abundance and diversity of AM fungal spores.
- To statistically assess any coincidence of AM taxon abundance and amendment treatment that may indicate nutrient-function efficiency.
- To statistically test if there may be any variation in spore abundance in the two disparate plant species that may indicate host-plant specificity or host preference.

4.3. Materials and methods

The field study was carried out during monsoon 2011. The study site (Plate 4.4a, 4.4b) was a 480 m^2 area over the the foredune ridge that includes *ca* 16 m of the the first two stations of the transect described in Ch. 3, and a *ca* 30 m perpendicular line over undulating ground following the N-S dune crest. Two clonal plant species were selected, the C_3 forb *I. pes-caprae* and C_4 grass *S. littoreus* that were co-dominant in the Ch. 3 survey (Table 3.1) The stolons of both species develop in all directions but particularly down the front slope into incipient dune (Plate 3.4). Neither species developed far into the backslope of the foredune in the study-site although interestingly, both species were observed to have spread respectively 30 to 35 and 60 to 70 m inland elsewhere in the dune system. *Ipomoea pes-caprae* is a deep-rooted (Plate 3.2), sand-binding, semi-succulent Na tolerant species (Suarez 2011), found in foredunes throughout tropical primary dune systems on coasts of five continents and most tropical islands (Hesp 2008). The species exhibits stomatal control of water loss and has low CO_2 assimilation rates primarily due to reduced chlorophyll concentrations per unit leaf area (Ripley and Pammenter 2004), and marked photoinhibition (Maun 2009). Propagation is achieved by prolific stolon development and ramet establishment, and abundant seasonal flowering and seed production. *Spinifex littoreus* is a perennial dioecious grass also restricted to coastal foredunes that has hirsute leaves resistant to saltwater spray. Its continental distribution is tropical Asia. Thick stolon-forming stems are frequently noded, from which copiously-leaved ramets develop abundantly, also deep-rooted and binding sand. Leaves are tough, inrolled, and with spiny tips. Both species are little grazed, *I. pes-caprae* leaves containing unpalatable toxins (Maun 2009) and sharp-pointed *S. littoreus* leaves acting as protective spines.

Plate 4.4a. Foredunes research site from the north. Arrow indicates focal point of **Plate 4.4b**.

Plate 4.4b. Foredunes research site from the south. Arrow indicates focal point of **Plate 4.4a**.

The experimental design was an arrangement of plant nutrient patches, RP, Zn and dry leaf-litter OM, separately and in all combinations, three replicates of each, placed beneath ramets of the two selected plant species showing root development, with controls, at the start of the monsoon rains, in a total of 48 plastic pots (75 mm diam., 60 mm depth, 240 mL capacity) inserted into the ground (Plate 4.1a, b; Plate 4.5). The pots were filled with plant-species specific rhizosphere soil that had been returned to the laboratory where additions of the mineral nutrients P_2O_5 at 40 µg g^{-1} in the form of rock-phosphate (RP) (UNIPHOS rock phosphate powder, 100 mesh fertilizer grade, 18% to 20% P_2O_5, manufactured Dec. 2008, M/S Unicorn Intercontinental Supplies, JayaNagar, Karnataka, India), and 24 µg g^{-1} granulated metal-Zn (99% minimum zinc metal powder, particle size 12-14 µm, Nice Chemicals Pvt. Ltd., Cochin, India), a mildly toxic level that may encourage plant uptake *via* AM fungi (Chen *et al*. 2003), were thoroughly and evenly mixed in by hand. Envelope sachets (mean 13.2 mL capacity [n = 7]) were constructed in the laboratory, made of 25 µm nylon membrane into which roots could not penetrate. Each was filled to capacity with crushed leaf-litter and sealed with Copydex® glue. Holes were drilled into the side and base of the pots to simulate drainage capacity of the surrounding on-site soils.

Rhizosphere soils had been separately collected to a 150 mm depth five days after the onset of monsoon rains from >25 genets rhizospheres of the two plant species and transported to the laboratory where each species was, discretely, thoroughly mixed by hand.. Three 50 g samples were taken from each mix and stored at 4ºC for later spore abundance analysis to genus level by the method described in Ch. 3. *Glomus* species sporocarps were counted as single units. Further soil sub-samples (100 g) were taken from each of the two soils before nutrient amendment, sieved to 2 mm and submitted to Goa Government Directorate of Agriculture, Soil Testing Laboratory, Ela Farm, Old Goa for soil chemical analysis. Methods

Plate 4.5. Partial-portrayal of the research site showing psammite soils, patchy distribution of the subject-plants, *I. pes-caprae* and *S. littoreus*, and extent of plant litter that contributes to soil OM. Arrows indicate pots positions in the topographically variable dune crest.

used for chemical analysis are described in General Materials and Methods. The weighed nutrient amendments RP and Zn were thoroughly mixed by hand with the respective rhizosphere soils and, along with filled sachets, returned to the study site. On site, stolons were cut apically at the selected ramets, the developing roots (*ca* 10 to100 mm long in *I. pes-caprae* and up to 150 mm in *S. littoreus*) carefully lifted, pots inserted to rim level with minimal disturbance, filled with the plant-species specific prepared nutrient-amended soils along with leaf-litter sachets (5.5% of pot volume) in OM treatments and, ensuring the developing ramet roots were firmly fixed in soils, left to be wetted by rainfall. Seven days later the stolons of both plant species were cut distally, making each subject a discrete unit.

Each insert was pegged and numbered. There were eight treatments: T1) unamended rhizosphere soils as control (C), T2) C + RP, T3) C + Zn, T4) C + OM, T5) C + RP + Zn, T6) C + OM + Zn, T7) C + OM + RP, and T8) C + OM + RP + Zn. All treatments and control were replicated three times. Pots were emplaced throughout the study site over the undulating foredune ridge (Plate 4.3a, 4.3b) non-randomly nine days after the onset of monsoon rains, ensuring replicates of each treatment were dispersed (Plate 4.5). No pots were placed beneath more than one ramet from the same genet. Ramets were monitored on a regular basis throughout the study period and shoot-growth recorded on days 32, 51, 72, 100 and 132. Length was summed where shoots were multiple. Mean length of each treatment was used in analysis. All other encroaching plants were constantly removed from within the pots and from close proximity at cotyledon stage. Root colonization was assessed on day 5 and day 52 of the study for presence/absence in nearby ramets only, thus confirming mycorrhization in each plant species but avoiding subject-pot disturbance.

The *S. littoreus* pots were carefully lifted after 132 days where all ramets had died by day 81. It was considered no further AM activity would occur after the intervening 51 days. *Ipomoea pes-caprae* pots were left for a further 40 days to encourage optimum sporolation in dried-out soils, before they too were lifted. All recovered pots were placed in polythene seal-bags, immediately removed to the laboratory and stored at 4ºC until processed. Root length, where it was possible to record data as samples were dry, brittle, and coiled in the pots, was a measure of the primary roots only. Fine roots could not be measured. Spore abundance pre-monsoon data were obtained by picking out all whole fresh spores from 3 x 50 g sub-samples from each of the collected *I. pes-caprae* and *S. littoreus* genets soils before addition of amendments, and post-monsoon data from 50 g sub-samples of each replicated treatment pot (n = 6 in *S. littoreus* and n = 9 in *I. pes-caprae*) when harvested, after sieving (355-38 µm)

and decanting (see General Materials and Methods). No protocol could be sourced in the literature for spore recovery from leaf-litter. Remaining leaf-litter in each of the replicate sachets from all treatments was wetted in DH_2O and, whilst agitating to release spores for extraction, carefully inspected in a glass Petri dish under the binocular microscope.

Statistics

Histograms were drawn in Excel 2007. Analysis of Variance (ANOVA) was carried out in Minitab 16. Significant treatment effects were characterized further with the Honestly Significant Difference (HSD) test at $P = <0.05$. Pearson's correlation was undertaken in the WebAgris 2.0 package, Indian Council for Agricultural Research (ICAR), significance tested by Soper (n/d).

4.4. Results

4.4.1. Plants

Ramet growth of both plant species in the emplaced pots was limited. *Spinifex littoreus* showed no shoot development in any of the treatments and all ramets had died 81 days into the study. *Ipomoea pes-caprae* growth (Plate 4.6) continued for up to a further 81 days

Plate 4.6. Shoot growth in *I. pes-caprae* sample I3C (*Ipomoea* treatment 3, C = the third of 3 replicates), after 100 days.

except in T1 (control) that had died soon after day 100, and T6 that had died by day 110. Growth declined as monsoon rains abated, and all had died before harvest. Figure 4.1 shows mean (n = 3) shoot growth in *I. pes-caprae* treatments, data recorded at five different time points over the season. ANOVA indicated significant increase over T1 in all treatments but T8 (Table 4.1).

There was no observable presence of intraradical AM fungal features in nearby ramet roots in either host species five days after onset of rains. On day 52 AM fungal colonization in *I. pes-caprae* ramets sampled near to the treatments pots was evident, both intraradical hyphae and vesicles observed (Plate 4.7a, b). In fine-roots of *S. littoreus* ramets growing close to

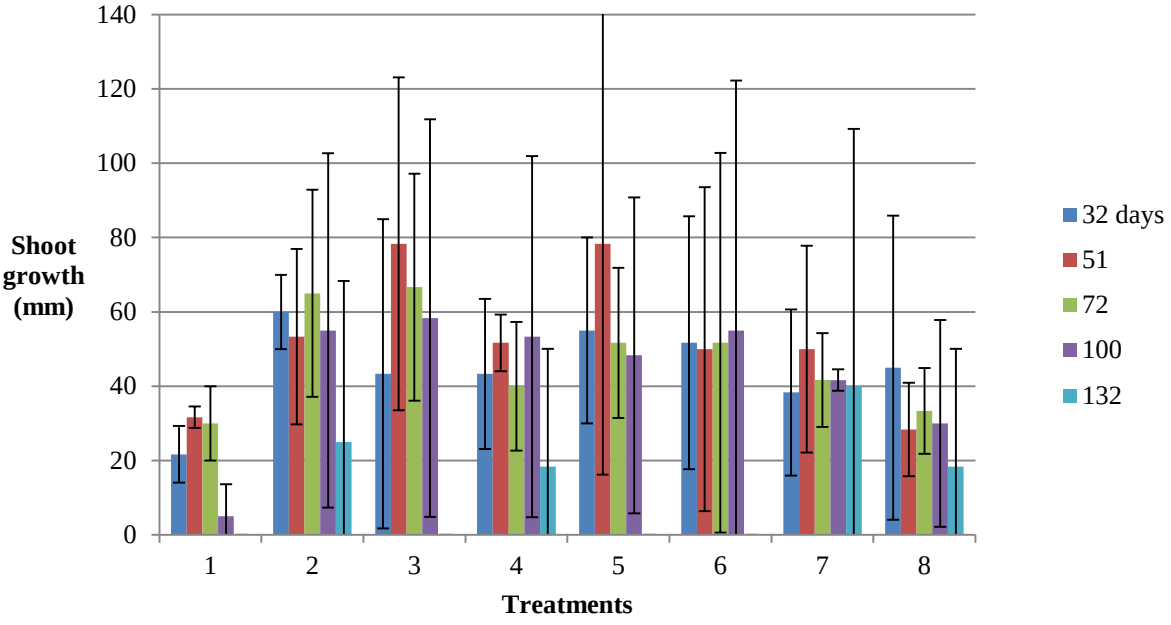


Fig. 4.1. Histogram of mean values of shoot growth of *I. pes-caprae* ramets in 8 amendment treatments at 5 time points over the experimental period (n = 3). All had died by day 141. Treatments were: T1. Control (C), T2. C+RP, T3. C+Zn, T4. C+OM, T5. C+RP+Zn, T6. C+OM+Zn, T7. C+OM+RP, T8. C+OM+RP+Zn. Error bars are SD about means.

Table 4.1. ANOVA of shoot growth of *I. pes-caprae* ramets controls (T1) *vs*. 7amendment treatments at means of 4* time points over the experimental period (n = 3).

	F	*P*
T1 *vs*. T2	28.47	0.002
T1 *vs*. T3	16.68	0.006
T1 *vs*. T4	12.52	0.012
T1 *vs*. T5	15.27	0.008
T1 *vs*. T6	22.31	0.003
T1 *vs*. T7	9.46	0.022
T1 *vs*. T8	2.66	0.154

T1 = controls, for T2-T8 treatments see **Fig. 4.1.** *Day 132 data is extraneous where growth in T1 is zero (**Fig. 4.1**). Shoot growth in all treatments excepting T8 was significantly greater than control. *P* = <0.05.

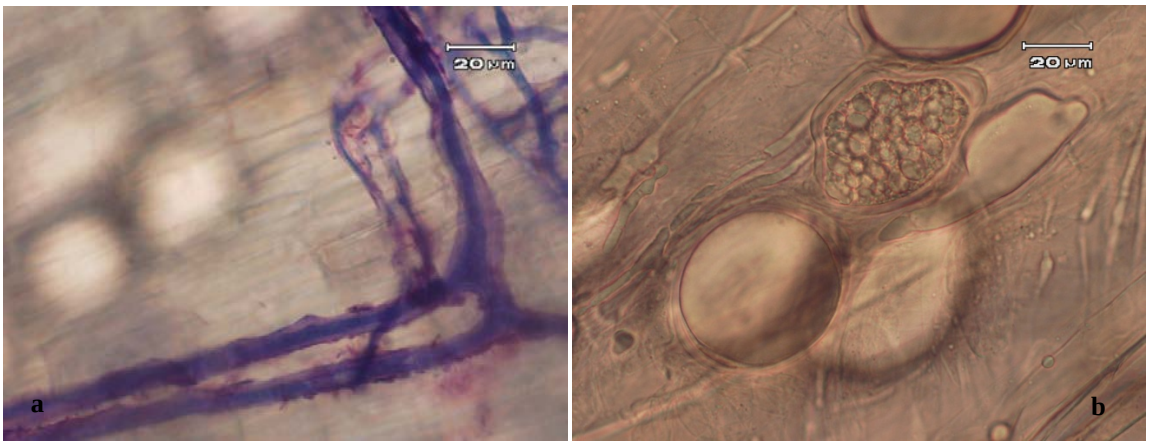


Plate 4.7a, b. Evidence of intraradical presence of AM fungi in *I. pes-caprae* plants sampled near to subject pots at day 52 indicating subject plants would also be colonized.

subject pots, all stained 1 cm root segments viewed were observed to be colonized by hyphae and vesicles (Plate 4.8a, b). Dried and brittle roots were coiled in the pots at the end of the

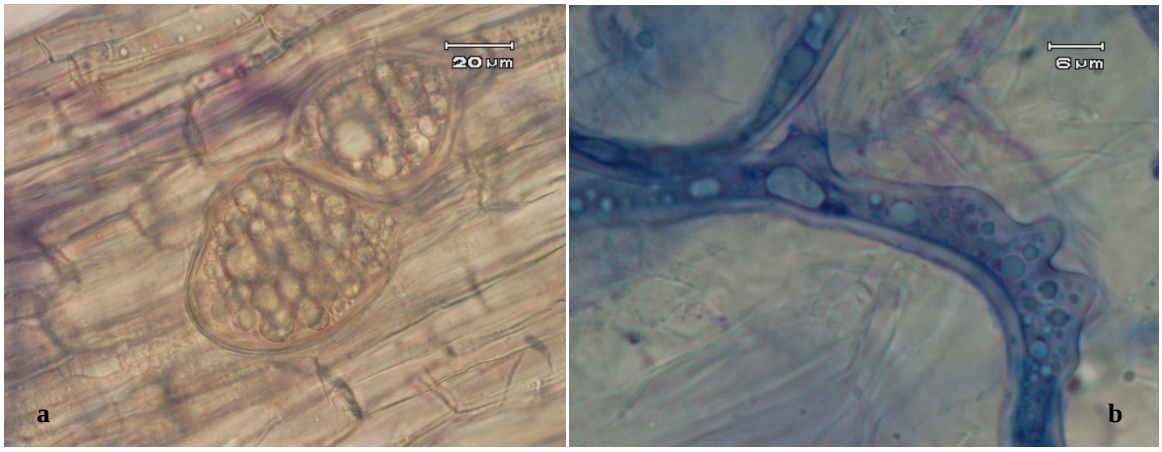


Plate 4.8a, b. Indication of intraradical presence of AM fungi in *S. littoreus* plants sampled near to subject pots at day 52. Colonization of subject ramets may not have been similar where all had died by day 60.

study period, lengths ranging from 25 to 61 cm in *S. littoreus* (n = 5) and 12 to 25.5 cm in *I. pes-caprae* (n = 10).

4.4.2. Soils analysis

Chemical analysis of genets soils extracted soon after onset of rains and before amendments addition (Table 4.2) indicated neutral pH in both host species, higher EC levels than transect

Table 4.2. Analyses of unamended rhizosphere soils from ramets of the two study-plant species.

	I. pes-caprae	*S. littoreus*
pH	7.0	7.1
EC dS m^{-1}	0.46	0.49
OM%	0.72	0.72
Tot N	0.072	0.072
P_2O_5 µg g^{-1}	7.32	traces
K_2O µg g^{-1}	10	10
Zn µg g^{-1}	3.168	1.256
Fe µg g^{-1}	8.24	8.48
Mn µg g^{-1}	3.98	2.06
Cu µg g^{-1}	traces	traces
B µg g^{-1}	0.29	0.18

Samples collected 9.6.11, from >25 genet rhizospheres of each plant species to 150 mm depth. Total N is factored from OM% (Singh, Chhonkar, and Dwivedi 2005).

survey means (Fig. 3.2b), low levels of OM, P_2O_5 and K_2O, higher than minimum plant requirement Zn and Fe and very low levels of Cu and B (Horneck *et al.* 1996). P_2O_5 concentration in *S. littoreus* soils was exceptionally low.

4.4.3. Spores

Spore abundance in soils collected from respective genet rhizospheres (pre-monsoon treatments T1) is shown in Fig. 4.2. *Acaulospora* species levels were 2.5x higher in *I. pes-caprae* than in *S. littoreus*, Gigasporaceae and *Glomus* at higher levels in *S. littoreus*. ANOVA indicates *Acaulospora* abundance is significantly greater in *I. pes-caprae* (F = 14.70; *P* = 0.019), there is no significant difference in Gigasporaceae between the plant species, and *Glomus* is significantly greater in *S. littoreus* (F = 480.50; *P* = <0.001).

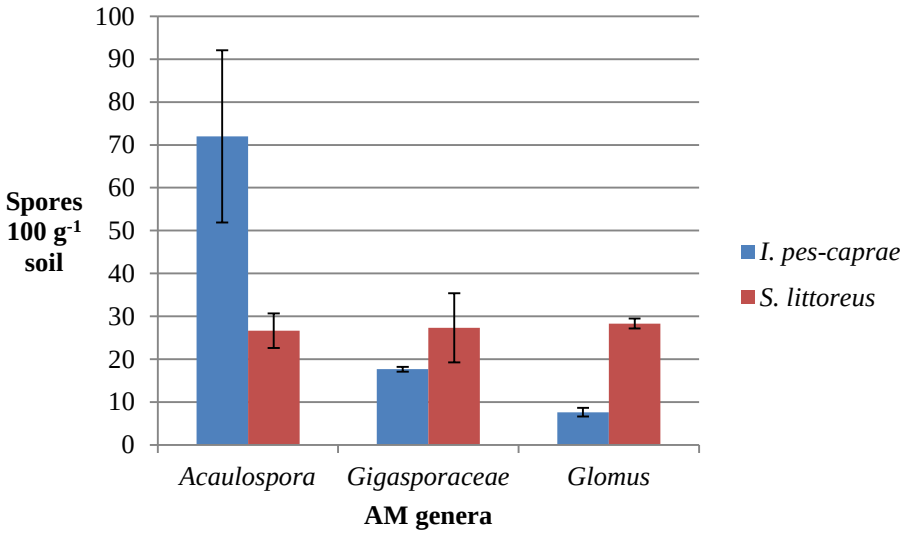


Fig. 4.2. Spore abundance in soil samples extracted at the beginning of the study period (26.5.11) before nutrient amendment.

Post-monsoon spore abundance and diversity in *I. pes-caprae* treatments are shown in Fig. 4.3, and in *S. littoreus* treatments in Fig. 4.4. ANOVA of pre-monsoon spore abundance in *I. pes-caprae* compared with post-monsoon T1 treatment indicates no significant difference in any of the AM genera, and in *S. littoreus* there is no significant difference in *Acaulospora*, significant increase in Gigasporaceae ($F = 9.35$; $P = 0.018$) and significant reduction in *Glomus* ($F = 30.08$; $P = 0.001$). ANOVA over treatments in *I. pes-caprae* indicates there is no significant difference between T1 (control) and any other treatment in *Acaulospora*, in Gigasporaceae T2 only is significantly greater ($F = 4.10$; $P = 0.001$) than control, and there is no significant difference between control and any other treatment in *Glomus*. Over treatments in *S. littoreus* ANOVA indicates there is no significant difference between T1 (control) and

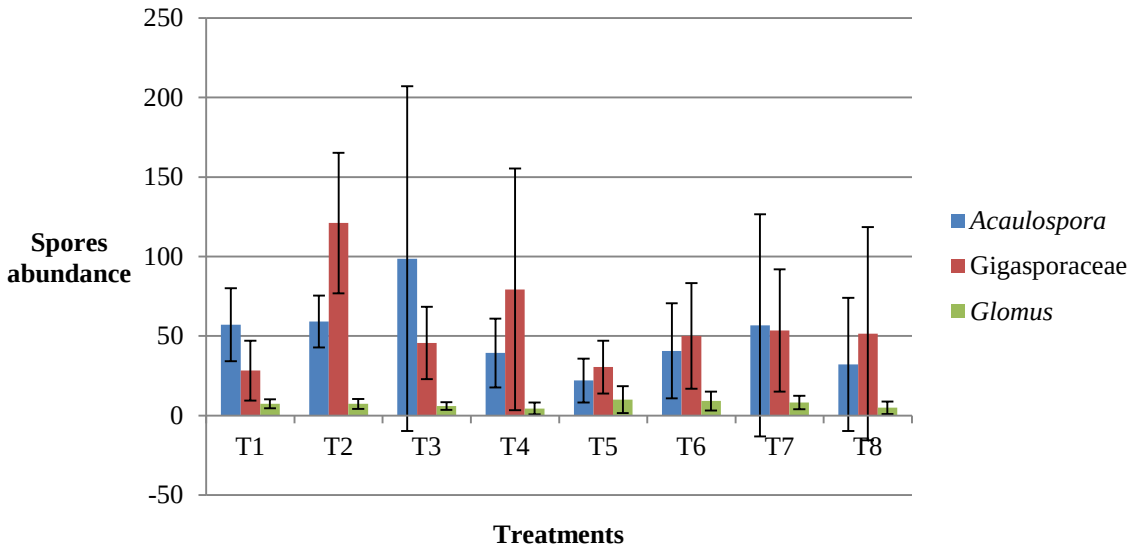


Fig. 4.3. Spore abundance (at genus level) recovered from *I. pes-caprae* treatments pots. Treatments were: T1. Control (C), T2. C+RP, T3. C+Zn, T4. C+OM, T5. C+RP+Zn, T6. C+OM+Zn, T7. C+OM+RP, T8. C+OM+RP+Zn. Error bars are SD about means (n = 9x 50 g samples).

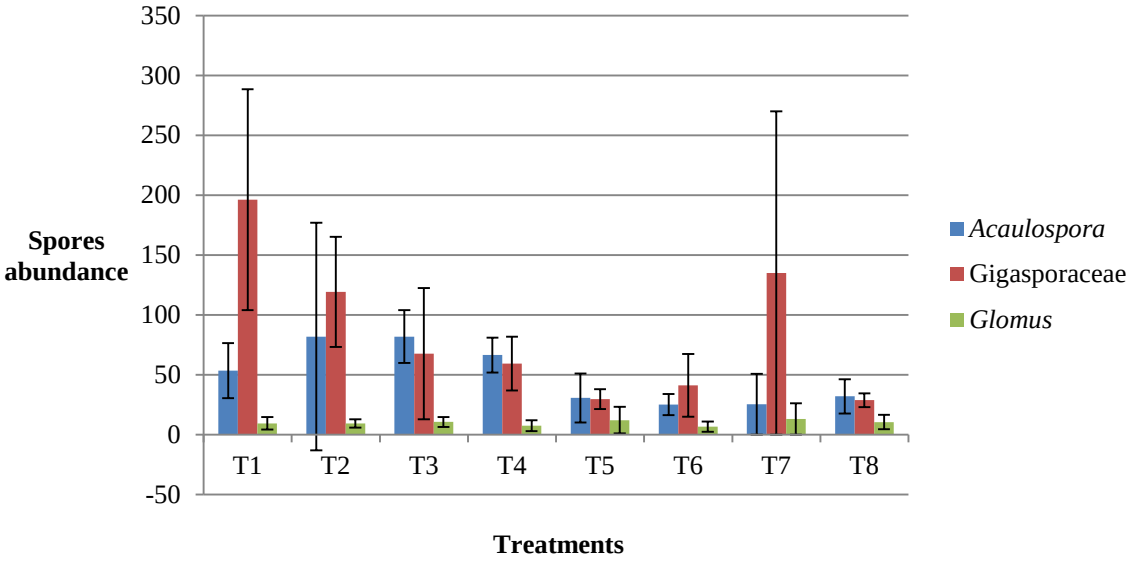


Fig. 4.4. Spores abundance (at genus level) recovered from *S. littoreus* treatments pots. Treatments were: T1. Control (C), T2. C+RP, T3. C+Zn, T4. C+OM, T5. C+RP+Zn, T6. C+OM+Zn, T7. C+OM+RP, T8. C+OM+RP+Zn. Error bars are SD about means (n = 6x 50 g samples).

any other treatment in *Acaulospora*, in Gigasporaceae control is significantly greater ($F = 10.81$; $P = <0.001$) than T3-T6 and T8, and there is no significant difference between control and any other treatment in *Glomus*. Groupings of Gigasporaceae spore abundance are shown in Table 4.3.

Correlation analysis showed no spore abundance consistency of AM taxa with nutrient amendment pattern (i.e. OM in T4, T6, T7, T8; P_2O_5 in T2, T5, T7, T8; Zn in T3, T5, T6, T8) in either host-plant post-monsoon dataset. Similarly, there are no significant correlations, positive or negative, between the two host-plant species.

The diversity in the spores recovered from leaf-litter sachets in both plant species included all of the taxa recovered from pots (Fig. 4.5, Fig. 4.6). ANOVA indicated there were no significant differences in AM spores abundance between treatments in either host, nor between hosts. Only two replicates, one each from *I. pes-caprae* and *S. littoreus* T7, contained no spores.

Table 4.3. ANOVA of Gigasporaceae spores abundance in all treatments, similarity groupings by Tukey's HSD test $P = <0.05$.

Treatments	Mean	Groupings				
S1	196.17	A				
S7	135	A	B			
I2	121.11	A	B	C		
S2	119.17	A	B	C	D	
I4	79.33		B	C	D	E
S3	67.5		B	C	D	E
S4	59.17		B	C	D	E
I7	53.56		B	C	D	E
I8	51.56			C	D	E
I6	50			C	D	E
I3	45.67				D	E
S6	41			C	D	E
I5	30.44					E
S5	29.5				D	E
S8	28.67				D	E
I1	28.22					E

Means that do not share a letter are significantly different. I = *I. pes-caprae* treatments, S = *S. littoreus*. Treatments were: 1. Control (C), 2. C+RP, 3. C+Zn, 4. C+OM, 5. C+RP+Zn, 6. C+OM+Zn, 7. C+OM+RP, 8. C+OM+RP+Zn.

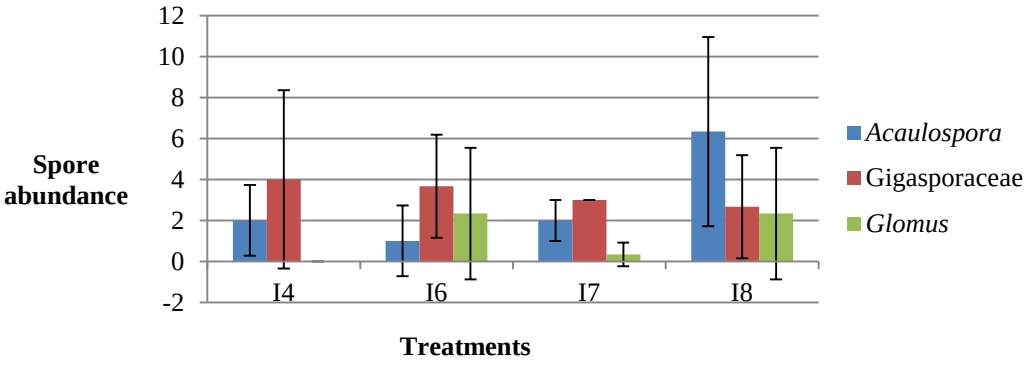


Fig. 4.5. Spores recovered from *I. pes-caprae* leaf-litter sachets post-monsoon.

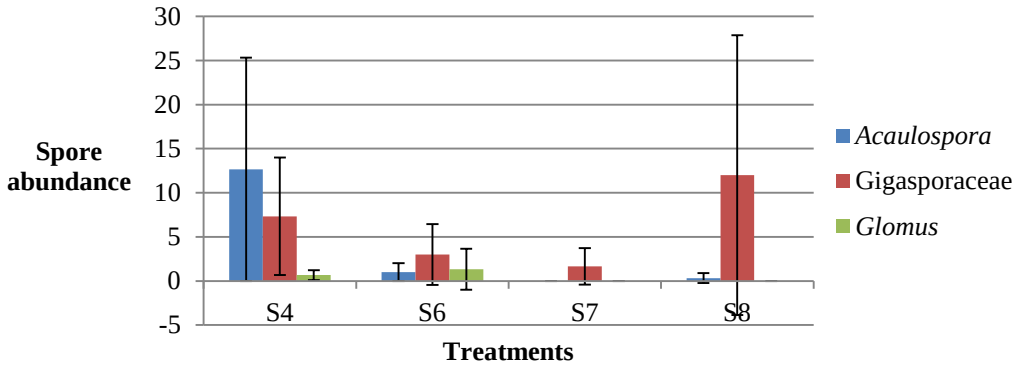


Fig. 4.6. Spores recovered from *S. littoreus* leaf-litter sachets post-monsoon.

Overall Gigasporaceae sum total was higher than that of *Acaulospora* in both plant species, in *Ipomoea* by 13.3% and in *Spinifex* by 70.8%. *Glomus* spores were more abundant in *S. littoreus* than *I. pes-caprae* in all treatments but T6. Sum total of *Acaulospora* in *I. pes-caprae* was 2.4% greater than in *S. littoreus*, and Gigasporaceae greater in *S. littoreus* by 46.4%. Sum total of all spores in *I. pes-caprae* treatments was 19.8% less than that in *S. littoreus*.

4.5. Discussion

Limited ramet shoot growth, and prevention of deep root-soil penetration, in both plant species in pots, suggests the cause of eventual ramet death in *I. pes-caprae* was restriction of access to deeper soil-water as monsoon rains abated. The same might be assumed of *S. littoreus* where roots similarly coiled in the pots were more than 2.5x greater in length than in *I. pes-caprae*. However, as there was no new above-ground growth observed in any *S. littoreus* ramet from the moment of seperation from the genet, senescence occurring in outer leaves within a few days (Plate 4.1b), it may be that *S. littoreus* ramets are more dependent upon resource from the genet during establishment than are those in an *I. pes-caprae* clonal community. Wilsey (2002) found connected ramet contribution to the clonal community led to higher overall biomass than that in severed ramets in Serengeti grasses and evidence from Hartnett and Bazzaz (1983) indicated new daughter ramets in *Solidago canadensis* L. (Asteraceae) are physiologically dependent upon resources translocated from their parental clone, dependency declining with time. The question of whether to cut or not had been considered beforehand. As a resource sharing strategy between a genet and its attached ramets where there is contrasting nutrient resource availability (Du *et al*. 2009) may reflect bias in direct ramet-isolate/AM fungal association, it was decided to sever. Response in the two plant species appeared to be very different.

Shoot growth in *I. pes-caprae* exceeded control in all treatments but T8, indicating positive response to all amendments applied singly and in combinations excepting where all three were simultaneously applied. Standard deviation (Fig. 4.2) within replicates in almost all treatments was large however, perhaps expressing high variability in the plant population, obscuring any AM nutrient-function efficiency trends there may be in the data. There were no conspicuous patterns relating shoot growth to either nutrient amendment, nor to AM spore

abundance or diversity. Nor was there any consistent relationship between any of the nutrient amendments and spore data.

Amendments increased soil P_2O_5 and Zn concentrations in the respective treatments to more than minimum plant-nutrient requirement of 25 to 40 µg g^{-1} of P_2O_5 (Bagyaraj and Balakrishna 2003, Sims 2000) and to mildly toxic levels of soil Zn (Chen *et al.* 2003, Takkar and Mann 1978). The P_2O_5 amendments should encourage decline in AM abundance where P becomes available to the plant *via* the direct pathway, and the Zn amendments promote proliferation of either enhanced-uptake function or phytotoxin-alleviation function taxa (Gildon and Tinker 1983, Christie, Li, and Chen 2004). Correlation of treatments with spore abundance and diversity showed neither increase nor decline that might be consistently attributed to AM response to either amendment however, even though Gigasporaceae abundance was significantly greater in *S. littoreus* control where P_2O_5 concentrations were notably low in pre-monsoon analysis (Table 4.2). This suggests nutrient function association, and functional efficiency. Organic matter amendment (5.5% by volume) also greatly increased soil levels beyond background (T1) levels (0.72%, Table 4.2), although not heterogeneous, but again there was no consistent correlation of AM taxa with the respective treatments. Spores recovered from leaf-litter sachets (Fig. 4.6, Fig. 4.7) similarly showed no consistent response pattern. Comparison at AM genus level may perhaps be too coarse a diversity index to indicate associations.

To find a greater overall spore abundance in *S. littoreus* ramets rhizospheres, where there had been no evident shoot growth, is perplexing. Arbuscular mycorrhizal fungi gain all of their C nutrition from host-plant photosynthate and consideration has to be made that there may have occured a rare example of AM fungal parasitism (Johnson, Graham, and Smith 1997), the fungi contributing to early death of ramets.

There were a number of experimental design errors evidenced during the study. Root-growth restriction in both host plant species undoubtedly mal-affected outcome and perhaps an open tube longer than the 60 mm depth pots used may have been a better container. This would have allowed deeper penetration of water-seeking tap roots whilst still confining the upper 20 cm of rhizosphere soil where the majority of AM function occurs (Oehl *et al*. 2004). Ramet severance from genets, and perhaps particularly in *S. littoreus*, may also have adversely affected normal development and in retrospect a clonal family nutrient-acquisition strategy, i.e. genet support of ramet growth and ramet contribution from amended nutrient availability, may have benefitted the study with a more consistent spore record. Also, the two plant species may have contrasting abilities in uptake and utilization of the three amendments. Ghannoum and Conroy (2007) described variation in P nutrition when comparing C_3 and C_4 grasses, for example. Furthermore it is possible that combinations of amendments may have had variable and even conflicting affect upon the symbiosis, positive and/or negative, that confounded a clearer outcome. The triple amendment combination in *I. pes-caprae* Treatment 8 was the only amendment that elicted no greater shoot growth than control (Fig. 4.2), for example. Shetty, Hetrick, and Schwab (1995) state plant growth may be impaired by Zn interference of P uptake by plants and Shuman, Dudka, and Das (2001) found biosolid compost soil amendments decreased plant availability of Zn, making it less toxic to plants, even in decreasing soil pH which increases Zn availability (Jeffery and Uren 1983).

Large deviation in both shoot growth and spore abundance data indicates considerable variability in interactions between plants, AM fungi and nutrient resource, and despite the consideration made of relative simplicity in the component parts enhancing a definitive line of enquiry, results have proved complex. Nevertheless, as a preliminary study, there has been positive outcome that may suggest further research strategy. Complexity might be

considerably reduced by limiting the number of experimental variables, even to the point of a single amendment in rhizosphere soils of a single plant species. Pot insertion into the rhizosphere matrix, with and without amendment, would seem to have been an effective method of gaining meaningful spore abundance and diversity data provided, that is, root biomass accommodation were to be better catered for, and taxa were classified to species level.

4.6. Conclusions

- *Ipomoea pes-caprae* shoot growth increased over controls in all nutrient amendment treatments excepting where all three were combined.
- All AM fungal taxa recovered were represented in leaf-litter OM sachets.
- Gigasporaceae was the dominant AM genus in both plant species.
- ANOVA indicated no significant difference in either AM fungal abundance or diversity between the two plant species.
- ANOVA showed no alignment of AM fungal taxa with any of the nutrient amendments in treatments, in either plant species.
- There was no indication of AM taxon-specific nutrient-function efficiency.

CHAPTER 5

A FIELD TEST OF AM FUNGAL FUNCTIONALITY: SPORE DIVERSITY AND DENSITY IN RELATION TO NUTRIENT AMENDMENT IN A UBIQUITOUS GRASS ALONG THE TRANSECT

Plate 5.1. *Ischaemum indicum* var. *indicum*

5.1. Introduction

Clear evidence of AM nutrient-function efficiency has not so far been found. Complexity, in what was conjectured might be a relatively simplified ecosystem, of both biotic and abiotic factors, and interactions between them, has proved to be far greater than anticipated. The transect survey, Ch. 3, has indicated there is no association of AM spore abundance with plant species distribution, suggesting the community compositions are independent of each

other. The survey also suggests AM fungi are driven by soil factors, and a number of possible nutrient-function associations between AM taxa and soil chemistry have been indicated. Negative association of *G. felinovii* with Na was discussed in Ch. 3 with the suggestion that where the taxon is most abundant on the transect, St. 5 and 6, a slightly elevated topography co-incident with slightly decreased Na concentrations (Fig. 3.2) may allow higher rates of sporulation. Table 3.4 further indicated positive association between *Gi. margarita* and pH, negative association of *S. calospra* with Na, negative association of *A. spinosa* with P_2O_5 and OM, and again negative association of *Gi. margarita* with P_2O_5 and OM. It is considered unlikely, and survey of the literature failed to discover any link, that pH gradient (Fig. 3.3) is the direct cause of *Gi. margarita* distribution, and hidden causation may be worthy of further investigation. The last two statistics seem anomalous and difficult to understand as the two fungal species spores are co-dominant over the transect up to the furthest inland station. Yet both OM and P_2O_5 mean concentrations are especially low (Fig. 3.2c, Fig. 3.2e) and deficient throughout, with temporal variation in P_2O_5 ranging from sufficiency to extreme deficiency (Fig. 3.2e), accentuating the complexity. The biology of AM symbiosis in a nutrient deficient soil would imply at least one of the two fungal species would facilitate uptake of either PO_4 or NH_4-N derived from OM (Mosse 1973, Hodge 2003, Smith and Read 2008). The Ch. 4 experiment data have not clarified the anomaly, there having been no consistent association of AM taxa with amendment treatments. It was notable however, that there had been a significant increase in Gigasporaceae spore abundance in *S. littoreus* control pots over all other treatments where P_2O_5 concentrations had been exceptionally low at the start of the monsoon season (Table 4.2).

At the end of the 2010 monsoon, *Acaulospora* and Gigasporaceae species were similar in abundance in St. 1 and 2, with a greater increase in *Acaulospora* species abundance during

the dry season before the onset of the following 2011 monsoon. Evidence from the Ch. 4 study indicated that at the start of the 2011 monsoon *Acaulospora* species were >3x greater in density in *I. pes-caprae* than in *S. littoreus* but the relationships were reversed in Gigasporaceae species, *ca* 20% lower in *I. pes-caprae* rhizosphere soils than in *S. littoreus*. At the end of 2011 monsoon Gigasporaceae abundance in *S. littoreus* control was 300% higher than in *I. pes-caprae*. This evidence suggests different host plant species may have variable affect on AM fungal spore abundance and diversity. Although there is little indication of specificity in the AM fungi/host plant association there is evidence of preference, a factor in which functional efficiency may be intrinsically involved. The experimental method described in Ch. 4, where pots-restricted root growth of the foredune clonal plants and where combinations of nutrient amendment may have obscured distinctive AM fungal spore response, was nevertheless profitable where there was indication of phytobiont/mycobiont preference.

It had been suggested in the Ch. 4 Discussion that a single nutrient amendment to pots with a single plant species that has a smaller root system, and AM spore abundance and diversity data characterized to species level, might reduce the complexity encountered in the foredunes experiment data. The plant species should also, perhaps, be non-clonal in habit. *Ischaemum indicum* (Houtt.) Merr. (Poaceae) (Merrill 1938) (Plate 5.1) is a perennial (USDA Natural Resources Conservation Service n/d) C_4 grass species (Collatz, Berry, and Clark 1998) distributed throughout tropical regions (Roberts 1970, Lu and Liu 2003, Potvini, Whidden, and Moore 2004). In this primary coastal dune system the species exhibits considerable plasticity by adopting an annual life-cycle (USDA Natural Resources Conservation Service n/d), propagating exclusively by seed. The species was observed to employ an ephemeral strategy, germination occurring soon after the onset of annual monsoon rains in early June,

the life cycle completed as soil-moisture availability declines during September/October and into November. A preliminary survey showed an expansive branching-pattern root system occupied a relatively shallow spatial niche. Primary root length was 45-110 mm (mean 77.6 mm: n = 17), foliar compliment along the entire length of transect remaining prostrate throughout the cycle. Flower stalks (culm) rise to 100-390 mm above ground level (mean 218 mm: n = 50) prior to seed formation enhancing dispersion. Interestingly prostrate culm formation was observed in grazed plants late in the season. Its distribution in the transect, as with all species recorded, is spatially patchy, but the species is ubiquitous to all stations (Table 3.1). Lowest frequency was in the region nearest to the foreshore at St. 1 and in almost all other stations it was the dominant species. Transect-wide soils physical and chemical characteristics, and AM spore abundance and diversity, have been extensively described in Ch. 3. There was indication of functional relationships between spore taxa and nutrient. *Ischaemum indicum* may thus prove a profitable candidate in which to introduce rhizosphere amendment in a further field experiment over the transect.

The selection of a single nutrient amendment option is not so obvious. The three added to rhizosphere soils in the foredunes, Zn, RP, and OM in the form of partially calcified leaf-litter (Ch. 4), had encouraged no clear pattern of alignment with AM fungal taxa across treatments. It was argued that Zn amendment had increased rhizosphere concentrations to a 'mildly toxic level' and thus would have had little effect on beneficial AM function, whether enhancing or inhibiting uptake. Increased concentration in amendment additions may trigger AM inhibition function, and thus present opportunity for greater sporulation, but there might be a number of other mechanisms involved in amelioration (e.g. Walley, Khan, and Bradshaw 1974, inherited resistance: Stoyanova and Doncheva 2002, succinate effect: Liang *et al*. 2007, Gu *et al*. 2012, silicon [Si] enhanced resistance). There may be further hidden factors that confuse

the outcome. A similar argument is proposed of AM acquisition function of many of the micro-nutrients (Liu *et al*. 2000, Göhre and Paszkowski 2006, Willis, Rodrigues, and Harris 2013).

Correlation evidence in Ch. 3 (Table 3.3, Table 3.4) has suggested the macro-nutrient (N, P, and K) concentrations in the soils are aligned with the OM and silt/clay fractions, and silt/clay fraction with OM fraction. It may be that the silt and clay particles are physically bound to the OM fraction forming soil-crumb aggregates (Christensen 1986), or that organic matter is adsorbed onto the clay and silt particles (Rennert and Mansfeldt 2003, Lūtzow *et al*. 2006). Hassink (1997) suggested clay and silt adsorption to be a microbial degradation protection mechanism, stabilizing OM in soils. There are considerable differences in structure (Skjemstad, Janik, and Taylor 1998), quality (Gregorich *et al*. 1994), and nutrient availability from (Kalbitz *et al*. 2000) OM, depending upon the type of origin material (Bending, Turner, and Jones 2002) and the extent of decomposition (Lützow *et al*. 2006). It was suggested in General Materials and Methods the analysed resource available at the start of monsoon season in the studied system is predominantly a recalcitrant OM material. It is composed mainly of root and foliage plant tissue that has been rapidly decomposed after having succumbed to the hot and dry environmental conditions that prevail between monsoons. The resource OM, N or dissolved organic matter (DOM), has been reported as the principal limiting factor to plant growth and succession development in primary dune systems (Olff, Huisman, and Van Tooren 1993) but is nevertheless, with silt and clay, a prominent source of nutrients during the rainy season in the study site. Phosphates desorbed by microbial acid- and alkaline-phosphatase activities, mineral phosphate solubilization (MPS), into the labile pool from Ca, Fe and Al cations would account for little available PO_4, even though Fe levels were found to be high in the rhizosphere soils of ramets investigated in the foredunes study

(Table 4.2). The literature suggests desorption occurs at soil pH 3.0 and less, similarly with Al (Kumar 2002). Kumar (2002) indicates phosphate desorption from Ca occurs at pH <5.0 but analysis of the dune soils indicates relatively low levels of Ca (see Fig. 3.2h) and may have little influence in total phosphorus nutrient availability. The biotic process, nevertheless, can contribute significantly to protonation of soil (Santi, Krishnaraj, and Alagawadi 2002), as does plant root exudation of organic acids (Hinsinger 2001). Micro-decomposer biomass-turnover may also make a contribution to trophic-level nutrition (Fermor and Wood 1983, Barron 1988). Skjemstad *et al*. (1992) found P correlated with Al-OM complexes in southern-Queensland dune sands.

Kalbitz *et al*. (2000) in their review suggest high microbial activity is positively related to DOM concentrations in soil solutions, and also suggest *ca* 10 to 40% of DOM may be easily decomposed by microbes. McCarthy *et al*. (1996) report the transport of strongly mineral-binding dissolved organic carbon (DOC) compounds from sandy soils under field conditions due to saturation of sorption sites. Soil-water saturation and warm soil temperatures experienced during west coast India monsoon may therefore have considerable significance in the availability of plant nutrient that is translocated from recalcitrant OM by AM fungi.

Arbuscular mycorrhiza fungal biology invariably facilitates nutrient uptake of N and P in nutrient deficient soils (Mosse 1973, Hodge, Campbell, and Fitter 2001, Smith and Read 2008). Thus the bulk of PO_4 nutrient, and N that can be taken up only from OM, sequestered by AM fungi in the dune system may be derived from OM complex. However, soil nutrient analyses indicated OM (Fig. 3.2c) and NH_4-N (Fig. 3.2d) remained consistently low spatially (even in the comparatively increased concentrations in St. 7) and temporally, whereas P_2O_5 (Fig. 3.2e) oscillated between severe depletion and adequacy over the monsoon season. Thus the plant demand for N *via* the AM indirect pathway would be constant, but for P inconstant.

This difference may reflect in taxon spore abundance. It was therefore decided to incorporate an organic material as the amendment to *I. indicum* rhizosphere soils throughout the transect, in the stations described in Ch. 3, a comparable highly calcified material to the indigenous material.

Vermicompost is just such a highly calcified organic material. It is a product of mesophilic bio-oxidative process in which detritivorous earthworms interact intensively with microorganisms and soil invertebrates within the decomposer community, strongly affecting decomposition processes, accelerating the stabilization of organic matter (Domínguez 2010, Lazcano, Gómez-Brandón, and Domínguez 2008). It is a finely divided peat-like material with a low C : N ratio, high porosity and high water-holding capacity, and may be a more readily-available nutrient resource than the leaf litter employed in the foredunes trial. Domínguez and Edwards (2004) suggest most nutrients derived from vermicompost are present in forms that are readily taken up by plants, and are thus equally readily taken up by AM fungal mycelia. Variability in the effects of vermicompost may depend on the cultivation system into which it is incorporated, as well as on its physical, chemical and biological characteristics (Lazcano and Domínguez 2011). The resource can vary widely depending on the original feedstock, the earthworm species used, the production process, the age of vermicompost (Warman and Anglopez 2010), and variation in plant species response (Roberts *et al*. 2007).

Effects of vermicompost on AM are reported in the literature. Kale *et al*. (1992) described a >300% increase in root colonization over controls in rice variety Hamsa. Cavender, Atiyeh, and Knee (2003) similarly found stimulation of AM root colonization in *Sorghum bicolor* (L.) Moench, at the expense of plant dry weight, when non-sterilized vermicompost amendment was applied. There was a general enhancement of dry weight in mycorrhizal

plants where sterilized vermicompost was applied. Sáinz, Taboada-Castro, and Vilariño (1998) on the other hand concluded high amounts of vermicomposted urban wastes might cause significant reduction in AM fungal activity. Clearly the biological relationship between AM, host-plant and vermicompost is as complex as every other aspect of AM ecology. Again a clear outcome in the experiment may be hindered. Nevertheless a transect-wide St. 1-7 (Fig. 2.2) AM fungal spore density and diversity comparison between vermicompost amendment and control at the species level, extracted from pot-contained rhizosphere soils of a single plant species, may further define, at fine scale, the nutrient role AM fungi play.

5.2. Objectives

- To assess variation in host-plant rooted density over the transect from previously recorded data.
- To assess the effect of vermicompost amendment to pot-contained rhizosphere soils of a single plant species along a transect, against controls, on AM spore abundance.
- To assess Simpson's diversity of AM from spore abundance.
- To relate variance in spore abundance to taxon nutrient-function efficiency.

5.3. Materials and methods

At the start of 2012 monsoon 25 µm nylon-membrane sachets into which roots could not penetrate (mean 13.4 mL capacity, n = 21, 5.58% pot volume) were constructed in the laboratory (as described in Ch. 4 Materials and methods) and filled to capacity with unsterilized worm-composted cattle manure, vermicompost that had undergone degradation for >2 years and hence was in a dry and friable, recalcitrant condition. The sachets were transferred to half of the alcohol-wiped sterilized pots (75 mm diam., 60 mm depth, 240 mL capacity) re-cycled from the Ch. 4 field experiment and placed beneath developing *I. indicum* seedlings in the transect stations investigated in Ch. 3 (n = 3 $St.^{-1}$), along with unamended controls (n = 3 $St.^{-1}$), on 4.7.12, 16 days after the onset of heavy monsoon rains. The seedlings selected at each station, based on visual assessment of vigour (up to second primary-leaf stage), each sample unevenly dispersed over station areas, were carefully transferred to the pots on-site along with their rhizosphere soils, the pots re-inserted into the ground to rim level at the points of extraction, and labelled.

The pots were removed after 131 days when only St. 7 pots had any remaining moisture after monsoon rain, transported to the laboratory and stored at 4ºC until processed. As some pots were not recovered, amended pots soils from each station were thoroughly mixed, from which 3x 50 g samples were extracted for spore abundance and diversity assessed to species level (see General Materials and Methods for procedure). Unamended (control) pots were treated in the same manner, here 1x 50 g sample only extracted for analysis. Vermicompost residues in sachets from amended pots were combined, each station composite carefully inspected and spores extracted by the procedure described for spore extraction from leaf-litter in Ch. 4 Materials and methods.

Root AM colonization levels were assessed by the Biermann and Linderman (1981) method from plant samples (see General Materials and Methods) in close proximity to pots (n = 5 St.$^{-1}$), collected on 29.7.12, on 12.9.12, on 26.9.12 when plants had initialized flower stalks, and on 1.10.12 as flowers and seeds were developing. It was not possible to gain data directly from pot-contained plants post-harvest, the roots having dessicated before the pots were retrieved. Plant frequency was assessed by random quadrat survey as described in Ch. 3 (n = 5 St.$^{-1}$) on 1.10.12. Root dry-weight was assessed post-harvest by combining the dried roots materials of each station, amended and control separately, and dividing total weight by the number of pots. A sample of the vermicompost was submitted for chemical analysis of pH, EC, OC, P, K, Mg, Ca and Na as described in General Materials and Methods before incorporation into sachets.

Statistics

Histograms, line-graphs and scattergraphs were drawn in Excel 97. All reference to correlation is Pearson's coefficient, significance tested by Soper (n/d). Diversity was assessed by Simpson's (1-D) index. Two-way ANOVA i.e. effects of station (distance from MH-WM) and vermicompost amendment, trend analysis, and principal components analysis (PCA) were conducted in Minitab16.

5.4. Results

Three amended pots and two controls were recovered from St. 7, similarly St. 5, all six pots from St. 6, 4, 3 and St. 2, and three controls and one amended only from St. 1. One vermicompost sachet from a St. 3 replicate had been breached by roots.

5.4.1. Plant frequency

Plant frequency across the transect (Fig. 5.1) was highly correlated (r = 0.927; *P* = 0.001) with the 2010 transect dataset. ANOVA indicated no significant difference in transect-wide means from those found in the survey conducted two years previously and described in Ch. 3. Frequency was similarly lowest in the foredunes, steadily increasing to 100% in the humic soils of the furthest station inland, St. 7. There was a contra-trend reduction in St. 5 in both datasets.

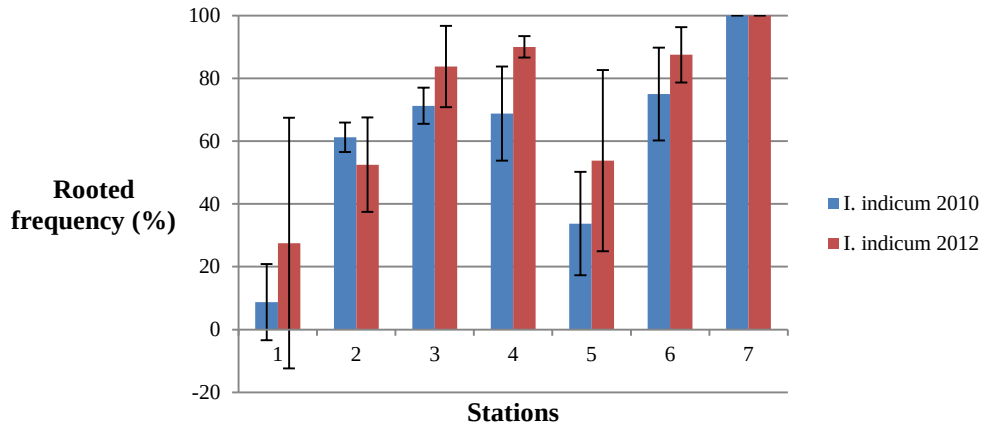


Fig. 5.1. Plant frequency of *I. indicum* in stations on the transect across the study site, data collected 20.9.10 and 1.10.12. Error bars represent S.D. n = 5.

5.4.2. Vermicompost analysis

Chemical analysis of vermicompost prior to installation indicates (Table 5.1) pH 6.0, high EC and OC% (OM%), P and K concentrations at adequate levels for plant growth by the

direct uptake pathway had roots been allowed direct contact, and high concentration of Mg and Ca. Sodium concentrations were slightly higher than mean levels indicated in the Ch. 3 transect survey (Fig. 3.2).

Table 5.1. Chemical analysis of vermicompost.

pH	6.0
EC dS m^{-1}	1.1
OC (%)	3.47
OM (%)	5.98
P µg g^{-1}	46
K µg g^{-1}	40
Mg µg g^{-1}	1440
Ca µg g^{-1}	3500
Na µg g^{-1}	75

Legend: OC% factored by 1.724 to give OM% (Singh, Chhonkar, and Dwivedi 2005).

5.4.3. Spore abundance and diversity

5.4.3.1. Genus level

Rank order total abundance at 'genus' (see description of classification used in the Thesis Pg. 48) level in amended and control pots was *Acaulospora* > Gigasporaceae > *Glomus* (Table 5.2a, b) with variable *Acaulospora* : Gigasporaceae ratio between stations (over distance) in each treatment, and between treatments. Gigasporaceae spores were consistently, and significantly ($F = 5.65$; $P = 0.004$) more abundant than *Acaulospora* and *Glomus* in sachets (Table 5.2c) in all stations. *Acaulospora* constituted 51.0% and 47.4% of the total in amended and control pots respectively, and Gigasporaceae 42.8% and 45.6% respectively. *Glomus* species accounted for 6.2% and 7.0% of the totals respectively. Gigasporaceae spores represented 87.9% of the total recovered from sachets.

Table 5.2a. Spore abundance in vermicompost amended pots at genus level.

Amended	St.	*Acaulospora*	Gigasporaceae	*Glomus*	Total
Pots	1	48.66	38	7.33	93.99
(100 g^{-1})	2	78	34	3	115
	3	106	149.31	4.66	259.97
	4	237.32	131.31	14.66	383.29
	5	221.99	211.66	17.33	450.98
	6	71.66	89.97	21.33	182.96
	7	19	2	26.65	47.65
	Total	782.63	656.25	94.96	1533.84

Table 5.2b. Spore abundance in control pots at genus level.

	St.	*Acaulospora*	Gigasporaceae	*Glomus*	Total
Controls	1	126	84	12	222
(100 g^{-1})	2	16	84	0	100
	3	134	218	6	358
	4	184	94	20	298
	5	16	123	0	139
	6	70	34	36	140
	7	118	2	24	144
	Total	664	639	98	1401

Table 5.2c. Spore abundance recovered from sachets in pots at genus level.

	No. recovered	St.	*Acaulospora*	Gigasporaceae	*Glomus*	Total
Sachets	1	1	2	18	0	20
	3	2	0	4	2	6
	3	3	8	240	0	248
	3	4	6	68	0	74
	3	5	22	158	0	180
	3	6	0	32	0	32
	3	7	18	60	22	100
		Total	56	580	24	660

Two-way ANOVA, station (distance) and amendment effects, on spore abundance in amended pots indicated no significant effect of either distance or vermicompost on *Acaulospora*. Neither was there significant distance effect in controls. In Gigasporaceae, station effect significantly increased (F = 5.49; *P* = 0.021: F = 11.83; *P* = 0.008) abundance in St. 3 and St. 5 respectively in amended pots. There was no significant amendment effect in any station. Nor was there significant distance effect on control pots abundance. There was significant (F = 5.06; *P* = 0.026) distance-effect increase of abundance in controls in *Glomus* in St. 6, but no significant effect, either distance or amendment, in vermicompost amended pots. There was thus no significant amendment effect on spore abundance in any of the genera.

Correlation of spore abundance in stations between amended pots soils, controls soils and residual vermicompost in sachets is shown in Table 5.3. There was positive correlation between sachets and amended pots soils in St. 3, between sachets and control pots soils in St. 3, and between amended and control pots soils in St. 1, in St. 3, and over the transect length.

Table 5.3. Correlation coefficient of spore abundance at genus level in vermicompost amended soils *v.* control soils, amended soils *v.* sachets, and control soils *v.* sachets in stations along the transect.

	Amended:Control	Amended:OM sachet	Control:OM sachet
	r	**r**	**r**
St. 1	**0.928** (*P* = 0.04)	0.532	0.550
St. 2	0.358	0.080	0.080
St. 3	**0.938** (*P* = 0.03)	**0.838** (*P* = <0.05)	**0.896** (*P* = <0.05)
St. 4	0.765	0.335	0.183
St. 5	0.504	0.574	0.865
St. 6	0.681	0.791	0.376
St. 7	0.326	0.209	-0.039
Overall	**0.902** (*P* = <0.05)	0.583	0.742

Legend. Bold figures indicate sig. correlation in stations. Amended treatment spores data are means of 3samples, control and sachet data of 1 sample.

There was no significant correlation of plant frequency with spore abundance in amended or control pots soils, or sachets.

5.4.3.2. Species level

Spores of 27 AM fungal species in four genera, *Acaulospora*, *Gigaspora*, *Glomus*, and *Scutellospora*, were extracted from amended rhizosphere soils, 19 from controls, and 14 from residual vermicompost in sachets. The total number of species recovered was 28. Eight species were recovered from amended pots not recovered from control. One novel morphotype recovered from residual vermicompost was recovered from neither amended nor control pots.

In amended pots (Fig. 5.2), *A. spinosa* accounted for 20.0% of the total of 1534 spores recovered, *A. scrobiculata* 20.8% (together 80.4% of all *Acaulospora*), *Gi. margarita* 23.1%, *S. gregaria* 15.5% (together 90.1% of Gigasporaceae), *G. felinovii* 4.4% and *G. claroideum* 1.2% (90.0% of *Glomus*), the six species together comprising 85.0% of the total spores recovered. Fig. 5.3a-f shows the distribution of these six species in transect stations. ANOVA indicates the distributions differ significantly in all six (*A. spinosa* $F = 8.38$; $P = 0.001$, *A. scrobiculata* $F = 9.13$; $P = <0.001$, *Gi. margarita* $F = 10.40$; $P = <0.001$, *S. gregaria* $F = 29.11$; $P = <0.001$, *G. felinovii* $F = 3.44$; $P = 0.027$, and *G. claroideum* $F = 15.69$; $P = <0.001$).

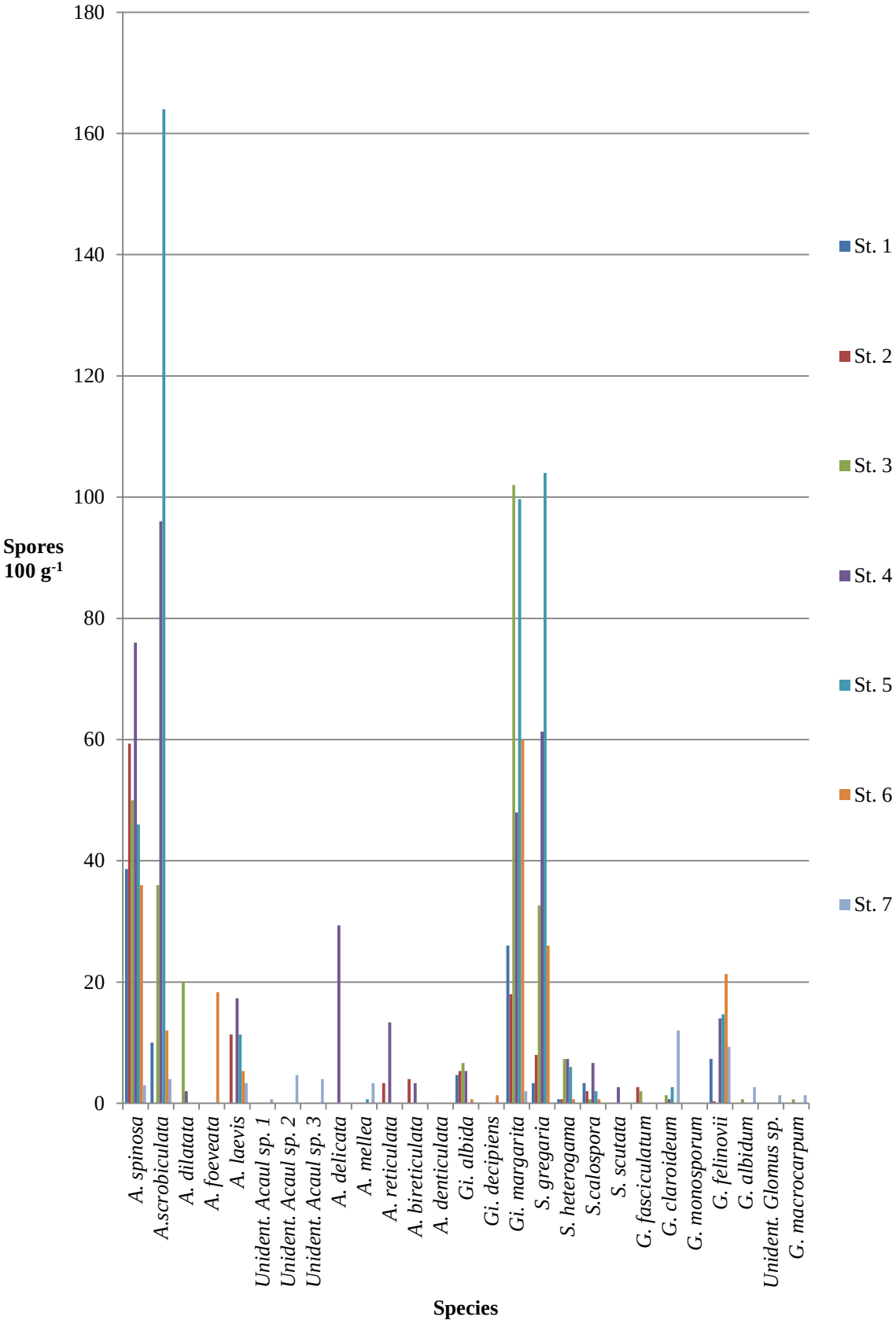


Fig. 5.2 AM fungal spore abundance and diversity in vermicompost amended pots.

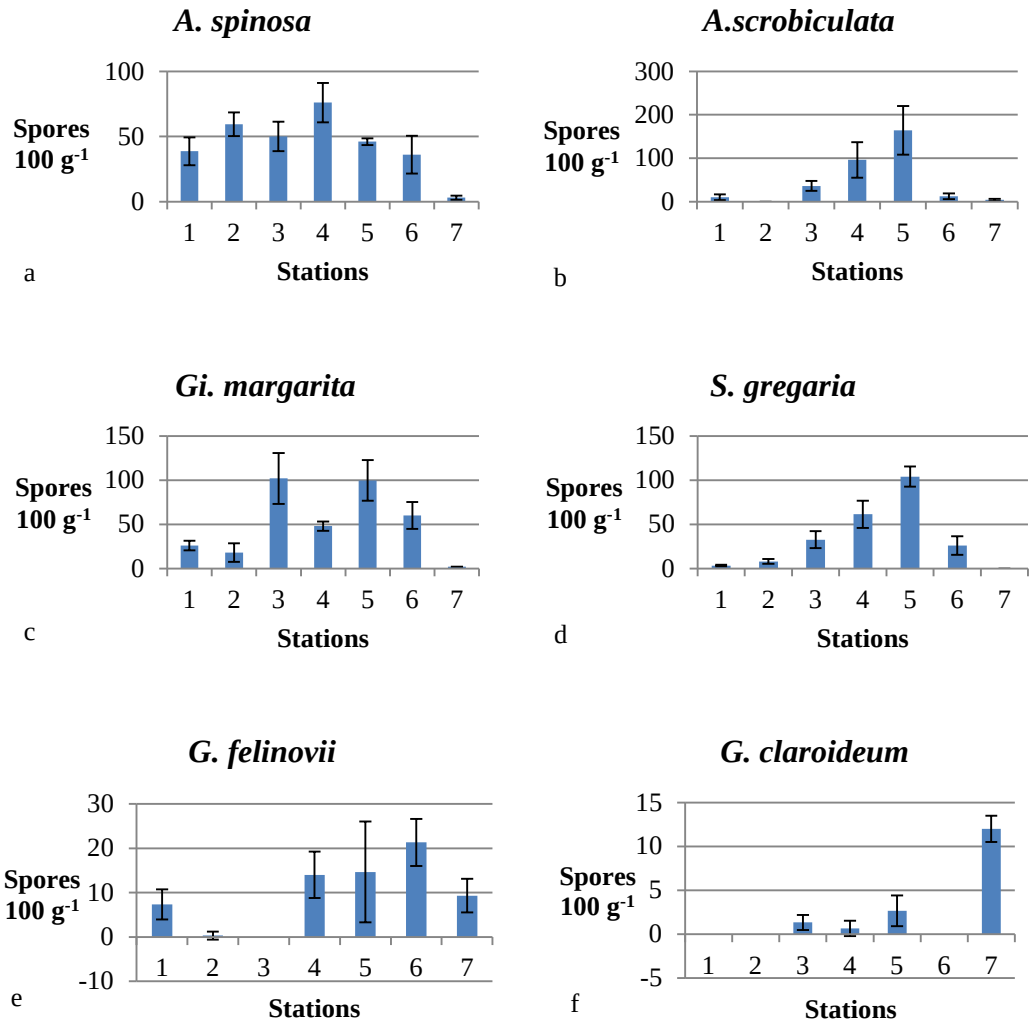


Fig. 5.3a-f. Dominant AM fungal species spores in vermicompost amended pots. Error bars = S.D. n = 3.

In control pots (Fig. 5.4) *A. spinosa* spores were 21.8% of the total recovered (1401), *A. scrobiculata* 12.1% (together 76.0% of all *Acaulospora*), *Gi. margarita* 30.1%, *S. gregaria* 7.6% (85.0% of Gigasporaceae), and *G. felinovii* 5.9% and *G. claroideum* 1.0% (together 98.0% of total *Glomus*), the six species together comprising 1100 spores (78.5% of the total).

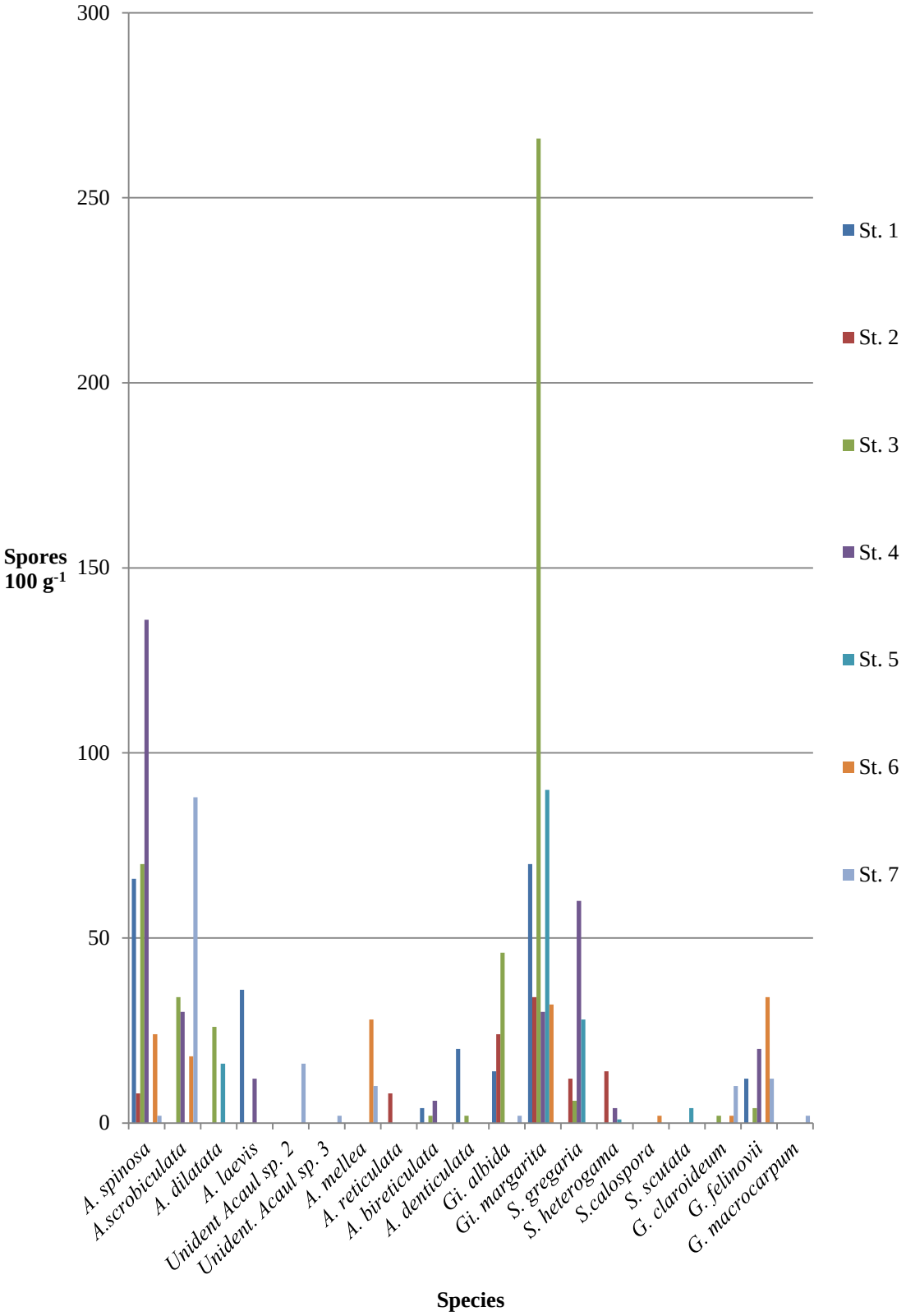


Fig. 5.4. AM fungal spore abundance and diversity in control pots.

Two-way ANOVA of treatment and station effects on amended and controls spore abundance at species level (Table 5.4) indicates *Gi. albida* was significantly affected by vermicompost amendment, and effect on *A. dilatata* and *A. denticulata* near-significant. There was no significant amendment affect on any other species, indicating the Thesis hypothesis of nutrient-function efficiency may be in jeopardy. Station effect was indicated in 16 of the 27 species, including the six co-dominant species common to amended pots and controls. Examination of these six species indicated (Table 5.5) abundance was significantly greater in St. 4 than in all other stations in *A. spinosa* amended, but significantly less (F = 8.38; *P* = 0.001) than in control. *Acaulospora scrobiculata* was significantly greater (F = 9.13; *P* = <0.001) in St. 5 than in all other stations in amended pots, and than in control. *Gigaspora margarita* and S. *gregaria* were significantly greater (F = 10.40; *P* = <0.001 and F = 29.11; *P* = <0.001 respectively) in controls than in amended over all stations (excepting St. 7 in *S. gregaria* abundance), *G. felinovii* significantly greater (F = 3.44; *P* = 0.027) in St. 6 and greater in controls overall, and *G. claroideum* significantly greater (15.69; *P* = <0.001) in St. 7 in both amended and control.

Table 5.4. 2-way ANOVA of station (distance) and amendment effect on AM spore abundance at species level in vermicompost-amended and controls rhizosphere soils.

AM Species	Station effect	Amendment effect
A. spinosa	**F = 5.83; *P* = 0.001**	**n/s**
A. scrobiculata	**F = 3.66; *P* = 0.013**	**n/s**
A. dilatata	F = 23.77; *P* = <0.001	F = 4.06; *P* = 0.058
A. foeveata	F = 2.97; *P* = 0.031	n/s
A. laevis	n/s	n/s
unident Acaul sp. 1	n/s	n/s
unident Acaul sp. 2	F = 6.35; *P* = 0.001	n/s
unident Acaul sp. 3	F = 5.27; *P* = 0.002	n/s
A. delicata	F = 8.81; *P* = <0.001	n/s
A. melea	n/s	n/s
A. reticulata	n/s	n/s
A. bireticulata	n/s	n/s
A. denticulata	n/s	F = 4.13; *P* = 0.056
Gi. albida	F = 2.64; *P* = 0.048	F = 7.52; *P* = 0.013
Gi. decipiens	n/s	n/s
Gi. margarita	**F = 19.82; *P* = <0.001**	**n/s**
S. gregaria	**F = 55.45; *P* = <0.001**	**n/s**
S. heterogama	F = 3.25; P = 0.021	n/s
S. calospora	F = 4.17; *P* = 0.007	n/s
S. scutata	F = 7.61; *P* = <0.001	n/s
G. fasciculatum	n/s	n/s
G. claroideum	**F = 24.15; *P* = <0.001**	**n/s**
G. monosporum	n/s	n/s
G. felinovii	**F = 5.25; *P* = 0.002**	**n/s**
G. albidum	F = 4.56; *P* = 0.005	n/s
unident Glomus sp.	n/s	n/s
G. macrocarpum	n/s	n/s

Species highlighted in **bold** are those co-dominant in amended treatment and control shown in **Fig. 5.3**.

Table 5.5. Comparison of spore abundance in amended and control rhizosphere soils of the 6 dominant AM species indicated in **Fig. 5.3**.

Station	*A. spinosa*		*A. scrobiculata*		*Gi. margarita*		*S. gregaria*		*G. felinovii*		*G. claroideum*	
	a	c	a	c	a	c	a	c	a	c	a	c
1	37	66	10	0	5	26‡	1	3‡	7	12	0	0
2	59	8	0	0	11	18‡	3	8‡	1	0	0	0
3	50	70	36	34	29	102‡	10	33‡	0	4	1	2
4	76†	136‡	96	30	5	48‡	15	61‡	14	20	1	0
5	46	0	164†	0‡	23	100‡	11	104‡	15	0	3	0
6	36	24	12	18	15	60‡	11	26‡	21†	34‡	0	2
7	3	2	4	88	0	2‡	0	0	9	12	12†	10†

Treatments: a = amended, c = control. † = sig. diff. in stations within treatment. ‡ = sig. diff. between treatments.

Spores extracted from vermicompost remaining in the litter sachets at the end of the season (Fig. 5.5) showed co-dominance of *Gi. margarita* (72.0%) and *S. gregaria* (17.2%). Fourteen species were recovered. One recovery was an unidentified and perhaps novel *Glomus* morphotype shown in Plate 7 (see **6.3. Recommendations for Further Work**) in high abundance (112 spores) from St. 5 only. The sample was not recovered from either amended or control pots rhizosphere soils. ANOVA indicates *Gi. margarita* abundance was significantly ($F = 4.44$; $P = <0.001$) greater than all other species in stations over the transect, other than St. 7, where none were recovered. The species was dominant (excluding the singular retrieval of the unidentified *Glomus* sp. described above) in all stations but St. 4 where *S. gregaria* was the dominant species. The two dominant *Acaulospora* species recovered from amended and control soils were greatly reduced (*A. spinosa* by 97% in both amended and control, and *A. scrobiculata* by 96% and 93% respectively) in abundance in sachets, *S. gregaria* abundance similar to control but less than amended, *G. felinovii*

recovered from St. 7 sachet only, and *G. claroideum* not recovered from any sachet. There was weak positive significant correlation in spore abundance between sachets and controls-soils spores over the transect (r = 0.742; *P* = 0.046). Correlation between sachets and amended pots soils was non-significant.

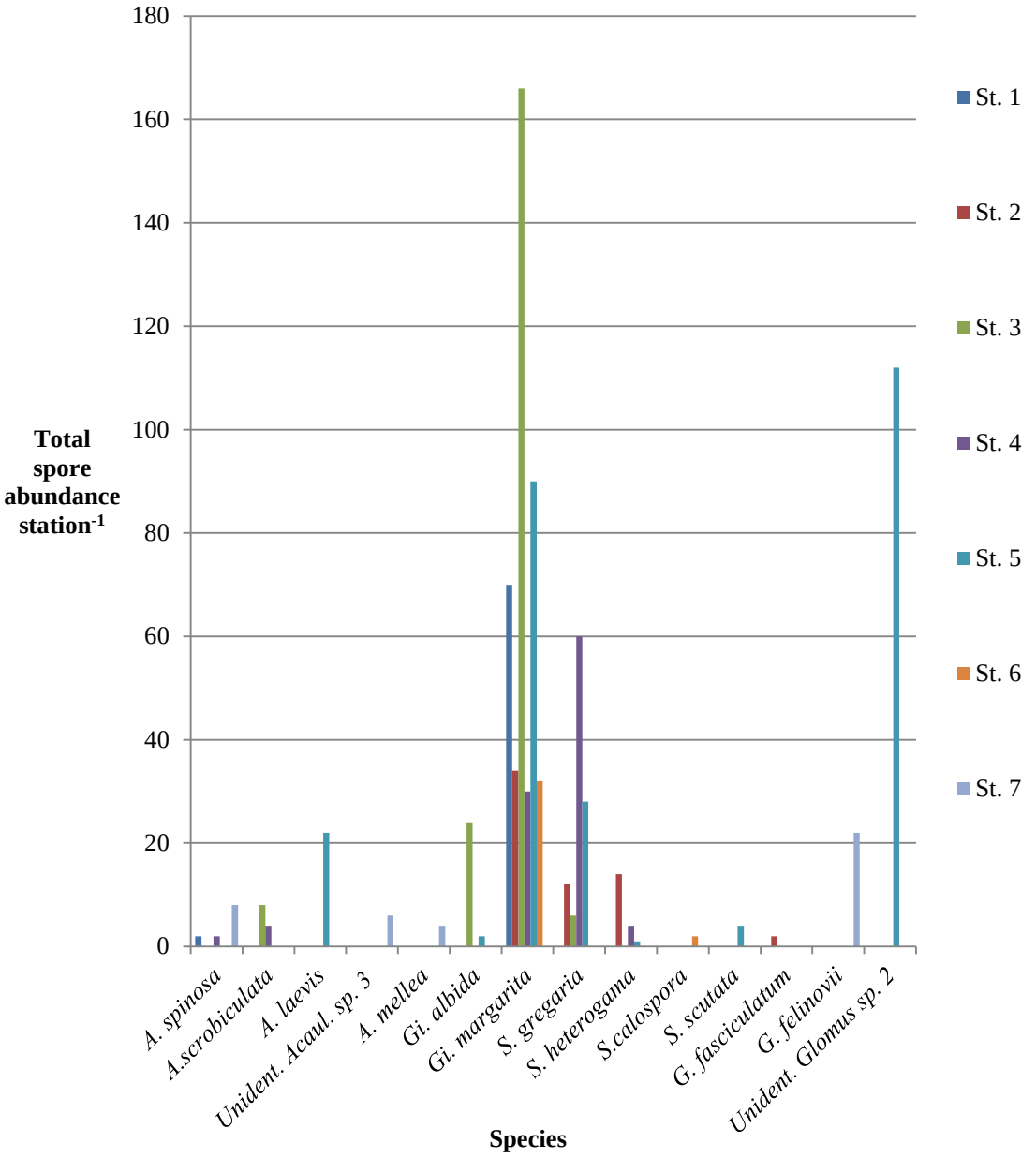


Fig. 5.5. AM fungal spore diversity and abundance in vermicompost remaining in sachets at the end of the monsoon season.

5.4.4. Root colonization

Intraradical colonization percentage (Fig. 5.6) by AM fungal structures (hyphae, vesicles and arbuscules) was highest, although variable between stations, during the juvenile plant vegetative phenological stage, falling at mature vegetative stage, increasing during culm development and falling again during flowering and seed development. There was no significant correlation between any of the four datasets. Neither was there significant correlation between 26.9.12 root colonization and plant frequency recorded on 20.9.12, nor between 1.10.12 root colonization, the final collection date, and any of the spore abundance datasets, amended, control and sachets, recorded after pot retrieval 12.11.12.

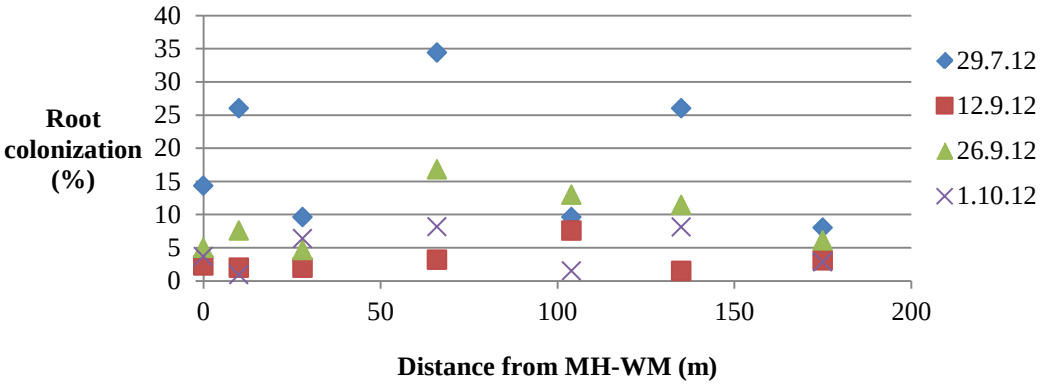


Fig. 5.6. Scatterplot of % AM colonization at 4 different times during the monsoon from fine-root sub-samples of 5 plants in close proximity to subject pots in each station. Data points correspond to St. 1-7 at 5, 20, 35, 65, 101, 138 and 175 m respectively.
Collection dates represent plant phenological stages**:** 29.7.12 = juvenile vegetative, 12.9.12 = mature vegetative, 26.9.12 = culm development, 1.10.12 = flowering and seed development.

5.4.5. Root dry-weight

Recovery of only one amended pot may have returned unrepresentative data in St. 1 and if excluded, the data (Fig. 5.7) suggest increase in root dry weight in amended pots over controls in all stations but St. 4 and 5. Two-way ANOVA indicates significant increase ($F = 6.34$; $P = 0.014$) over the transect (omitting St. 1) but no signicant effect of amendment.

Correlation analyses was carried out between root dry weight in amended and control pots, and spore density in amended, control and sachets, to assess any indication of relationship between root biomass and spore density. The analyses showed only a significant negative correlation (r = -0.927; *P* = <0.001) between amended pots roots dry weight and amended pots spores abundance.

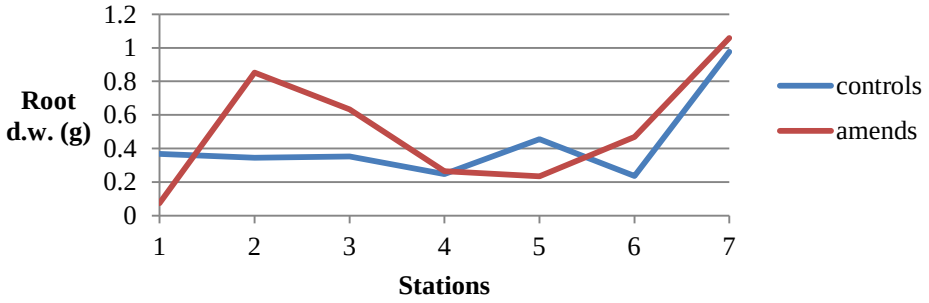


Fig. 5.7. Root dry-weight from amended and control pots at the conclusion of the study. Roots from retrieved pots (3 amended and 2 controls from St. 7, similarly St. 5, all 6 pots from St. 6, 4, 3 and 2, and 3 controls and 1 amended from St. 1) were weighed as the total in each station and divided by the number of pots for each treatment.

5.4.6. Simpson's diversity of AM spores at species level

Simpson's index of diversity of species-level spores in amended pots, control and sachets at each of the stations on the transect and overall transect are shown in Table 5.6. Over the whole length of the transect there is little difference in amended pots and control, 0.836 and 0.875 repectively, with sachets exhibiting the least diversity of all. Controls and sachets were equally diverse, and greater than amended, in St. 1 and St. 2, the trend reversed in St. 3 to St. 7 where the vermicompost amended pots consistently displayed greater spore diversity (Fig. 5.8). Where there was significant positive correlation between amended and control pots spore abundance over the whole transect (r = 0.902; *P* = 0.049), correlation of diversity was negatively non-significant. There was significant positive correlation between diversity in

amended pots and 1.10.12 rooted frequency (r = 0.941; *P* = <0.001). ANOVA indicates there is no significant difference between the three datasets over the transect, and two-way

Table 5.6. Simpson's index of diversity of AM spores at species level in amended and control pots, and sachets.

	Amended	Controls	Sachets
St. 1	0.494	0.774	0.758
St. 2	0.697	0.806	0.800
St. 3	0.766	0.717	0.375
St. 4	0.847	0.727	0.585
St. 5	0.753	0.528	0.693
St. 6	0.838	0.808	0.484
St. 7	0.921	0.601	0.609
Overall	0.836	0.875	0.443

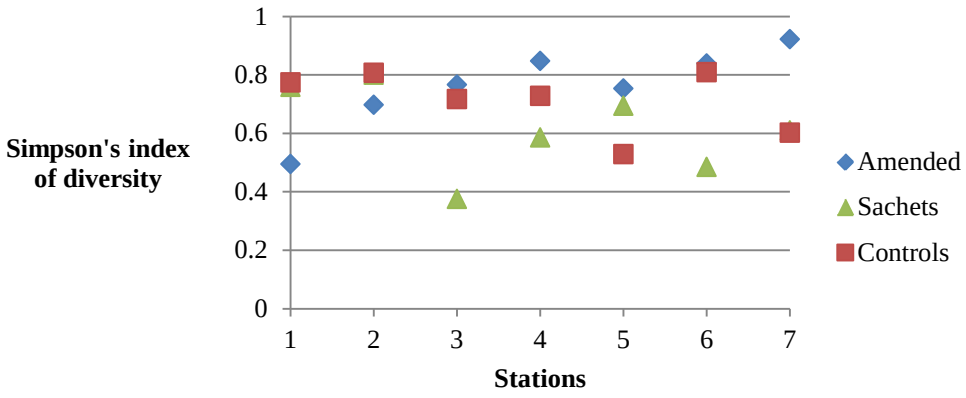


Fig. 5.8. Scatterplot of Simpson's index of diversity of AM spores at species level in amended and control pots, and sachets.

ANOVA indicates no significant effect of station treatment in any of the sets. PCA of distance *v.* Simpson's diversity of spores in amended and control pots (Fig. 5.9) indicates discrete species distributions in each of the two treatments.

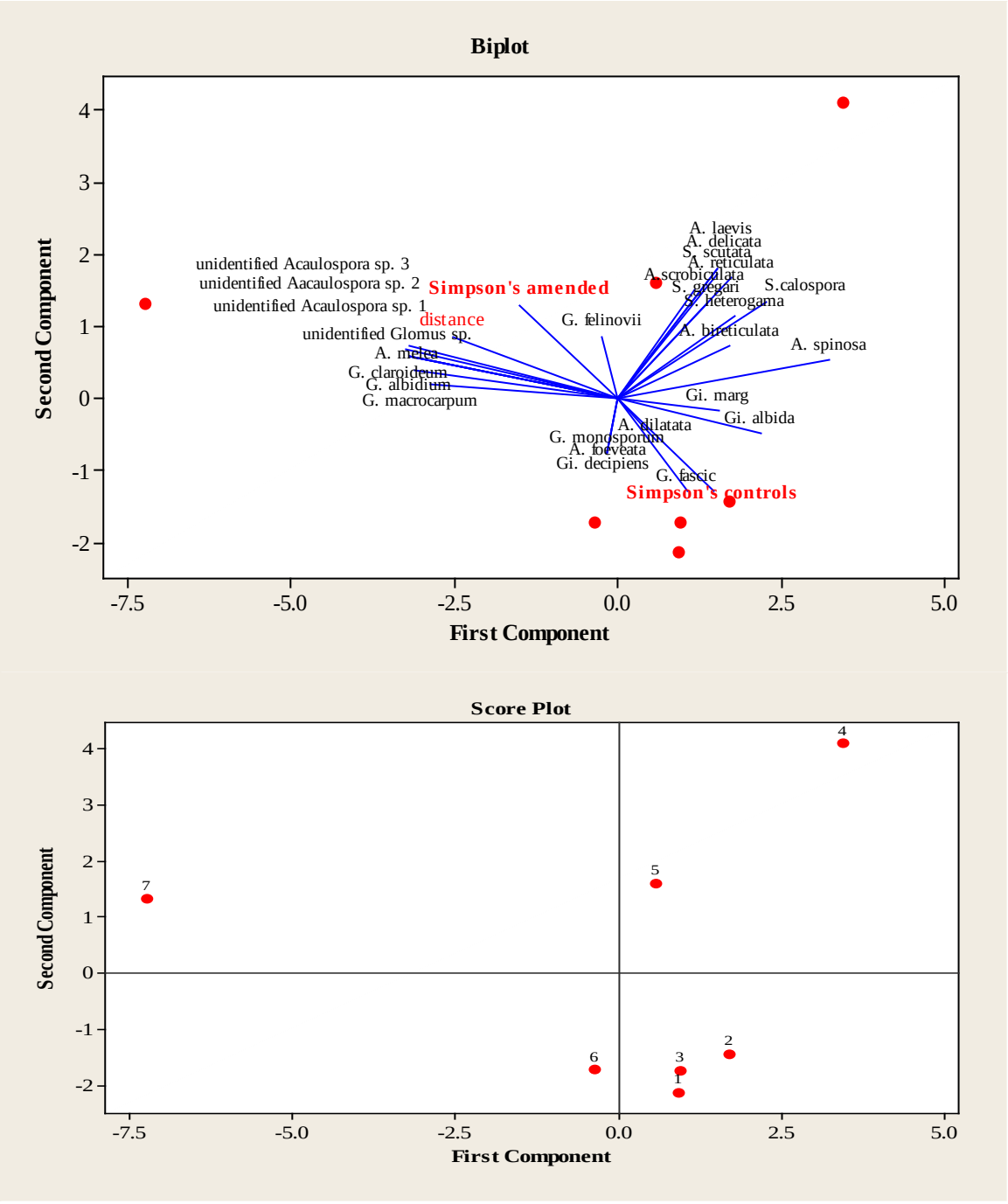


Fig. 5.9. Biplot and Score Plot of PCA of Simpson's diversity of AM spores in amended and controls against distance over the transect. The two axes jointly explain 59.3% of the variation.

A scatterplot of Simpson's diversity in amended and control pots against distance over the transect (Fig. 5.10) shows variation in controls but suggests an increasing trend in amended pots diversity. Trend analysis (Fig. 5.11) supports this.

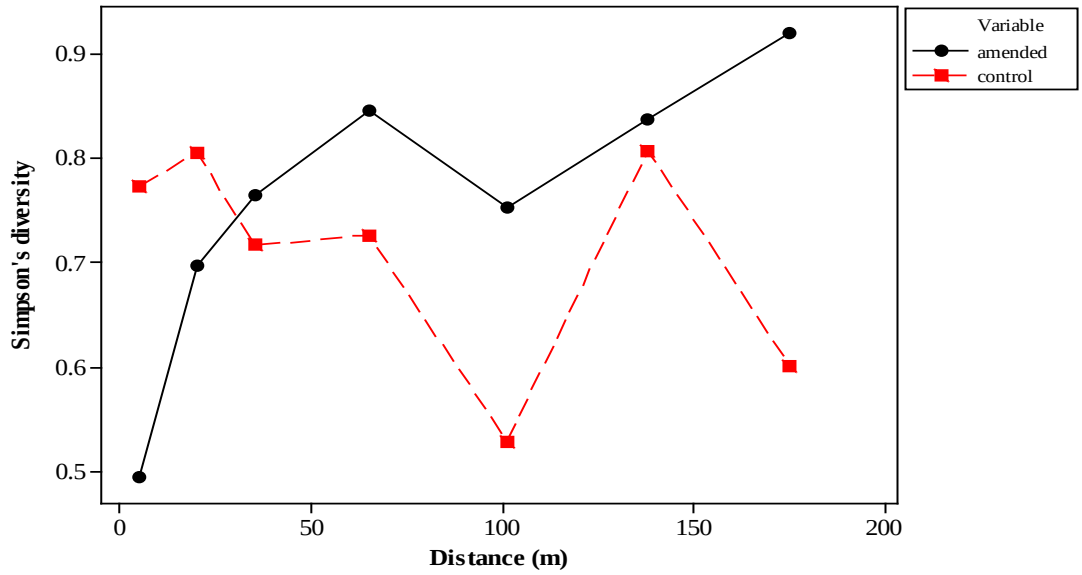


Fig. 5.10. Scatterplot of Simpson's index of amended and control treatments spores at species level over the transect.

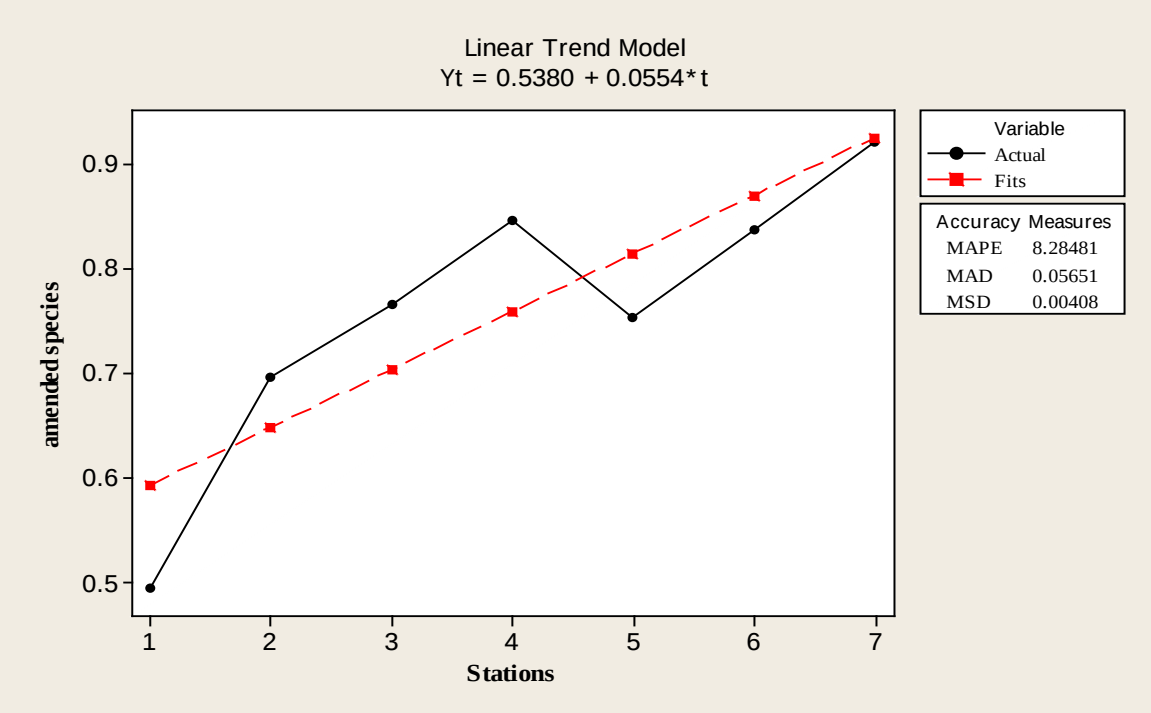


Fig. 5.11. Trend Analysis Plot for Simpson's diversity of spores in amended pots. The mean absolute % error (MAPE), mean absolute deviation (MAD) and mean squared deviation (MSD) accuracy measures are all small, indicating a robust fit (Hobai 2009)

5.5. Discussion

The experimental design, simplified from that in Ch. 4, appears to have returned more robust data.

5.5.1. Plant frequency

Host plant frequency along the transect was closely aligned to the dataset from the initial survey made in 2010 (Fig. 5.1). Only St. 2 showed greater frequency in the earlier set. St. 7 frequency was 100% on both occasions. Strong correlation between the two transect-length datasets, and no significant difference in ANOVA, suggests that the plant community component of the study is, overall, relatively stable from year to year, and may indicate a system-wide stability despite landscape change being the rule rather than the exception in coastal dunes (Provoost, Jones, and Edmondson 2011, Arens *et al*. 2005). Further comparative data over a number of seasons should be gained to confirm this however.

5.5.2. Vermicompost amendment and roots

Root colonization and dry weight data make no evidential contribution in support of the Thesis hypothesis. They do, however, substantiate other aspects of AM biology that are often reported. Temporal variation in root colonization that aligned with host growth and development is indicated in Fig. 5.6. The highest levels, and those with greatest variation between stations, were recorded in early stage of plant development, 25 days after the onset of heavy monsoon rains, 13 days after planting out. The lowest levels were evident at the end of the vegetative stage (70 days). Levels increased as flower stalks rapidly developed and fell again as flower and seed developed. ANOVA, however, indicated no significant transect-wide differences between levels. There are reports of colonization level variation along with the host plant growth stage (Hartnett *et al*. 1993, Lugo, Maza, and Cabello 2003, Pongrac *et*

al. 2007, Koide 2010). The evidence presented here may be in agreement. Root dry weight was unaffected by the addition of vermicompost in sachets. The significant negative correlation between amended pots root dry-weight and amended pots spore abundance is in agreement with changes in root architecture that are commonly reported to occur upon colonization by AM fungal hyphae i.e. reduced length, reduced biomass, fewer root hairs, coarser roots (Fitter 1991, Hetrick 1991, Atkinson, Berta, and Hooker 1994, Berta *et al.* 1995, Smith and Read 2008).

5.5.3. Vermicompost amendment and spore abundance

Vermicompost sachets comprise *ca* 5.5% of amended pots by volume, considerably greater than indigenous soil OM concentrations of <0.05%-1.5% (Fig. 3.2), but the material not heterogeneously distributed through the pots soils. Interestingly directional root growth toward sachets was observed in a number of the pots. Chemical analysis (Table 5.1) indicates a nutrient-rich readily-available resource that, contained in 25 µm membrane, can be directly accessed by AM fungi but not by roots. The directional root growth is, however, indication of response to a nutrient diffusion gradient from sachets, a nutrient(s) that is mobile in the soil continuum (Brady 1974, Casper and Jackson 1997, Farrar *et al.* 2003). There is no evidence to indicate which nutrient(s) that might be. The literature suggests N may readily diffuse through the soil-water continuum (Kurtz, Melsted, and Bray 1952, Cornforth 1968) and that P and K may be rapidly fixed in soil particle and are relatively immobile (Hinsinger *et al.* 2011). Organic P may be more labile than inorganic P (Fernandes and Sanford 1995). These differences in lability may have considerable implication in AM fungal hyphal nutrient-uptake biology. The phylum is particularly adept in facilitation of N and P uptake (Smith, Grace, and Smith 2008, Smith and Smith 2011), it is suggested especially so in a warm and wet low-nutrient environment such as tropical-monsoon coastal sand dunes.

Despite variance indicated by 2-way ANOVA in station (distance) effect, spore abundance in amended and control-pots soils showed notable consistency in percentage ratio of genus totals over the transect. *Acaulospora* abundance in amended was just 3.6% greater than control, Gigasporaceae and *Glomus* in amended pots both less than control (2.8% and 0.6% respectively). Significant Pearson's correlation supports the statistic. Whether this is a further indication of system stability cannot be ascertained on the evidence provided and, as with plants frequency, further comparative data over a number of seasons should be accumulated to verify the phenomenon. Contrary to the thesis hypothesis, 2-way ANOVA also indicates there was no significant amendment effect on spore distribution at genus level.

Spore abundance in sachets was dominated by Gigasporaceae in all stations, >80% of total spores recovered. This strongly suggests there had been association preference on the part of either host plant, or fungal species, or both, in the sequestration of nutrient from vermicompost. Total transect abundance of Gigasporaceae spores in sachets (580) is similar to amended (656) and control (639). In *Acaulospora* however, there were 783 spores in amended soils, 664 spores in control, and a total of only 56 in sachets. Clearly there is differential response to vermicompost amendment in the two genera. The greatly reduced sachet total in *Acaulospora* may be a significant feature. Whether that difference is due to Gigasporaceae having greater functional efficiency in the sequestration of any one particular nutrient sorved fro vermicompost cannot be ascertained from the evidence. It is likely, however, the species is efficiently facilitating uptake of N, or P, or both of these nutrients.

Vermicompost addition is further seen to have impacted AM fungal diversity at the finer species-level scale. Analysis resulted in 27 species recovered from amended pots soils, 19 from control, and 14 from sachets, one of which was not recorded in either amended or control soils. The same two co-dominant species in each of the three genera was a common

feature in amended and control pots soils spore abundance. Rank order was reversed only in the two dominant *Acaulospora* species, *A. spinosa* and *A. scrobiculata*, on one occasion in the anomalous St. 5. High incidence of the *Acaulospora* species, in both amended and control, and the Gigasporaceae species *Gi. margarita* and *S. gregaria* in control soils, suggests fitness in the association with *I. indicum*, and robust hierarchical trophic-level position. It can only be assumed that these factors may relate to functional efficiency. Significant difference in distribution of the six species over the transect stations may be an indication of variation in intolerance to, or greater functional ability in, varying soil environment along the transect. *Glomus claroideum* for example, was limited to the more acidic humic soils of St. 7 (Ch. 3) albeit at low abundance. *Acaulospora spinosa* displayed more of a generalist (see Glossary) strategy, accommodating environmental variation from St. 1 up to St. 6. Two-way ANOVA of amended pots spore abundance against control indicates significant amendment effect in one species only, *Gi. albida*. There is thus no evidence to suggest there may be species variation in AM nutrient-function efficiency.

Gigaspora margarita spores were dominant in residual vermicompost in sachets, 64.2% of total recovery, and *S. gregaria* 16.1% of the total. Other than the unidentified *Glomus* morphotype uniquely encountered in St. 5 sachets, the remaining 11 species were represented at comparatively low abundance. Of particular note is the virtual absence of *A. spinosa* and *A. scrobiculata*, both of which had occurred at high abundance in amended and control pots soils. It can only be concluded that the *Acaulospora* species, despite evidence of high MIP in control and amended pot soils, found no advantage in invading the sachets. Gigasporaceae species on the other hand, particularly *Gi. margarita*, readily sporulated in sachets (Table 5.7). Why this phenomenon should occur is not clearly evidenced, but it is reasonable to

assume that the Gigasporaceae fungi accessed the OM to gain nutrient, and high spore abundance, in *Gi. margarita* in particular, suggests the species functioned efficiently.

Table 5.7 indicates further, but anomalous, dominant *Acaulospora* and Gigasporaceae relationships. There is parity in spore abundance of *A. spinosa* in amended and control soils, at high density. This suggests vermicompost amendment has had no effect on *A. spinosa* abundance, which virtually no sporulation in sachets corroborates. There is greater abundance (almost 2x) of *A. scrobiculata* in amended over control, also at high density. This suggests vermicompost addition has affected increased abundance but, again, there is virtually no sporulation in sachets. There was a singular instance of *A. scrobiculata* abundance equivalence in amended pots (20.7%) that may indicate significant increase in response to vermicompost, but predominantly in St. 4 and 5 pots only and, inconsistently, relatively few spores in the other stations.Neither *Acaulospora* species has invaded the sachets to any great extent to capture nutrient.

Table 5.7. Comparisons of spore abundance of the dominant *Acaulospora* and Gigasporaceae species in vermicompost-amended and control pots, and residual sachets material. Data are totals of St. 1-7.

AMENDED			
A. spinosa	*A. scrobiculata*	*Gi. margarita*	*S. gregaria*
309	322	355	235
CONTROL			
A. spinosa	*A. scrobiculata*	*Gi. margarita*	*S. gregaria*
306	170	522	106
SACHETS			
A. spinosa	*A. scrobiculata*	*Gi. margarita*	*S. gregaria*
12	12	402	96

Conversely, there is greater abundance (1.5x) of *Gi. margarita* in control over amended, suggesting sachet amendment has had little or no effect, yet in sachets the species is dominant, almost 65% of all spores recovered, 77% of the two *Acaulospora* and Gigasporaceae species combined. If high spore abundance is an indication of 'fitness of the species' (Pg. 21) then the 'C-for-nutrients exchange' model of the symbiosis dictates *Gi. margarita* has made considerable nutrient contribution to the host plant. Lastly there is greater abundance of *S. gregaria* spores in amended over control (2.2x), suggesting vermicompost addition has had a significantly positive effect. Density in sachets was slightly less than in control, indicating the species had also contributed nutrient to host plant directly from vermicompost, but perhaps to a lesser extent than *Gi. margarita*.

Which nutrient(s), and taxon specific functional efficiency in delivery of particular nutrients (if the Thesis hypothesis is tenable), cannot be ascertained from the data.

The phenomena may be explained by the insoluble nature of P that, in the vermicompost nutrient analysis, is indicated to range up to 46 µg g^{-1}, a concentration high enough to support plant function by the direct pathway. The sachet material however, excludes plant roots, and as little P may diffuse through the sachet membrane into rhizosphere soil, it may be the host plant is reliant upon AM fungal association for P nutrient acquisition from vermicompost. This suggests the Gigasporaceae species are intrinsically involved in indirect pathway P uptake. Similarly, if N is diffused from sachets into rhizosphere soil, and the *Acaulospoa* species are particularly efficient in N facilitation to host, there would be no need to invade sachets to maintain fitness. The reasoned hypothesis may be supported by data from the single sachet (St. 3) that had been breached by roots where no *A. spinosa* spores were recovered, eight out of total 12 from all stations *A. scrobiculata* spores were recovered, 16 of 96 *S. gregaria* spores, and almost 50% (194 of 402) of total *Gi. margarita* spores were

recovered. The argument is tenable, and may indicate a future avenue of investigation into taxon specific nutrient function efficiency.

5.5.4. Simpson's index of diversity (1-D)

Simpson's index of spores at species level, except in the first two stations on the transect, was greater in amended than in control pots. This is partly explained by the increase in total spore abundance in amended pots over controls and partly by more even distribution, but also by eight fungal species extracted from amended soils not found in controls, and one species found in controls that was not found in amended soils, albeit at low density. A causal relationship between Simpson's diversity in amended pots and rooted frequency from quadrat survey indicated by significant positive correlation ($r = 0.941$; $P = <0.001$) seems unlikely and this may simply be a statistical artifact. ANOVA indicates there is no significant difference between the three datasets (amended, control, and sachet) over the transect, and no significant effect of station, nor treatment, in any of the sets. Again there is no evidence to support the Thesis hypothesis. Principal components analysis of spore diversity in amended pots and control indicates the same distinct partition along the transect as that described in Ch. 3 where pH and OM and spore abundance and OM correlations identified partition between St. 1-3 and St. 4-7. Trend analysis, after comparing spore abundance in amended and control pots along the transect, indicates an increased species diversity from foredunes to furthest inland station.

5.6. Conclusions

- Plant frequencies over the transect were highly similar in the two datasets, the more recent generally increasing by 9.5-18.75%.
- Amendment with vermicompost as an OM resource in the rhizosphere soils of a single plant species over the transect has impacted the abundance and diversity of AM fungi.
- *Gi. margarita* sporulation was notably high in residual-vermicompost sachets suggesting functional efficiency.
- There was an increasing Simpson's AM diversity gradient over the transect related to vermicompost amendment.
- Simpson's spore diversity in amended pots and control suggests there is distinct partition in the transect, St. 1-3 and St. 4-7.
- There was no evidence to indicate AM taxon specific nutrient-function efficiency.

CHAPTER 6

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER WORK

The more I look, the greater the wondrous complexity I see.
Willis 2013

6.1. General Discussion

Data analysis has indicated significant trends and relationships both expected and surprising within soils, and between soils, plants and AM fungi.

The plants on the transect are strongly affected by wind-shear (see Plate 6.1) that contributes to the prostrate habit described, a common strategy that helps reduce transpiration and therefore desiccation (Davies, Gill, and Halliday 1978, Huang and Fry 1999, Ripley and

Plate 6.1. Comparative *I. indicum* growth in wind-exposed (**A**) and wind-sheltered (**B**) environment.

Pammenter 2004). Offshore winds velocity is high enough to transport the smallest sand-grain particles from the strand along the transect to at least St. 4 (65 m) before there is any decline in matrix <52 µm sand-grain percentage content. Plant zonation has been attributed to sand-particle aeolin movement and consequent plant burial (Moreno-Casasola 1985 and references therein, Dech and Maun 2005), by Na concentrations (Mariko *et al.* 1992, Wilson and Sykes 1999, Maun 2009), and extent of adapted tolerance and specialization to the hostile coastal dune environment. Except immediately before onset of heavy monsoon rains, Na concentrations in soil were not at a level great enough to be deleterious to plants that are adapted to dune habitat (<55 µg g^{-1}). Even though CCA suggests otherwise, Na still may have had some effect on plant zonation, particularly in the foredunes, the region most prone to sea-water spray, and where 'specialist' species *I. pes-caprae* and *S. littoreus* are dominant. Sand grain mobility and displacement were not assessed.

There is a decreasing pH gradient along the length of the transect from St. 1 in the foredunes to St. 7 at the furthest point inland, a feature commonly found in coastal dune systems (Lichter 1998, Lane *et al*. 2008, Gormally and Donovan 2010). A second and interesting gradient indicated increase in AM fungal diversity along the transect in vermicompost amended *I. indicum* pots that cannot be a direct response to the amendment. Pots in all stations had the same volume of vermicompost incorporated. A further transect-wide influence, it is suggested, has affected diversity but correlation with pH indicates only a weak significant negative relationship ($r = -0.758$; $P = 0.048$). Whether pH is a direct or indirect cause of, or even involved in, increasing AM spore diversity cannot be concluded from the evidence.

The pH gradient is significantly negatively correlated with OM and P_2O_5, there is part-transect strong negative correlation with NH_4-N, and lastly significant negative correlation (r

= -0.797; P = 0.016) with transect-survey mean plant frequency. This is consistent with the model of a pedogenesis process associated with increasing organic material, humus accumulation, over distance inland that is commonly reported in dune systems (Bonte and Hoffmann 2005, Buurman and Jongmans 2005, Buurman, Jongmans, and Nierop 2008). Increasing plant root density promotes greater organic acid exudation into the rhizosphere (Maun 2009, Schaaf *et al.* 2011) that, along with bacterial and fungal decomposer organism activity (Maun 2009), decreases pH. In turn mobilization of phosphate and nitrate in recalcitrant soil organic matter increases. Increasing soil acidification (range pH 7.0 to pH 3-4) was reported by Nierop and Verstraten (2003) to have increased lignin degradation in recalcitrant coastal dunes soil organic matter, for example. The labile pool thus affords greater available nutrient to further increase plant density, and so on. The process, according to the evidence, is gradual up to the abrupt change recorded at the furthest inshore point on the transect (St. 7) where rubification has occurred. This colour change indicates a transition from psamment inceptisol to humic mollisol soil (USDA NRCS n/d), described above as a pedological transition zone. The argument is equivocal however. Tipping and Woof (1990) calculated that an increase in soil pH of 0.5 units in the range pH 3.3-5.5 would lead to increases of about 50% in the amount of mobilized organic matter in organic soils. Kalbitz *et al*. (2000), in their review, state “Almost all laboratory studies show that dissolved organic carbon (DOC) release from organic soil horizons is positively correlated to pH” but later point out that data from field studies may not support these observations, decreasing pH observed to have increased and decreased DOM release. You, Yin, and Allen (1999) reported increased OM dissolution when pH increased.

The spatial abruptness of the transition at St. 7, as far as is known, has not been previously reported. Whether this is an evolutionary, and perhaps unique response to the India west-

coast monsoon environment, or an anthropogenic phenomenon cannot be ascertained. Historical evidence would suggest the Portuguese agricultural administration, if the increase in fertility is a natural occurence, converted the dune system to paddy after the innermost station on the transect. It may also be, however, that decades or perhaps tens of decades of domestic animal grazing practices that, it was observed, were most prevelant in this higher plant density region, may have enhanced, or been wholly responsible for, increase in N input through defecation. Comparisons would need to be made with other similar dune-systems or, if it were possible, a domestic animal exclusion experiment conducted.

In retrospect it is considered that percentage OM calculated from OC levels recorded in samples that have been sieved to 2 mm, so removing larger fragments of organic material, is a crude and conservative estimate. Although results are deemed accurate, and correlation between partial NH_4-N data estimated by the Indian Bureau of Mines titration method and OC estimated OM by the Walkley and Black (1934) method clearly indicates similarity ($r = 0.9998$), an additional loss on ignition or combustion method (Read 1921) may have returned a more accurate and relevant determination of total % by volume. Furthermore, analysis could then perhaps have been undertaken of a finer rhizosphere sample scale which may have addressed resource heterogeneity, patchiness that obviously must spatially affect AM fungal dynamics (Dowding 1959, Nicolson 1959, Koske, Sutton, and Sheppard 1975, Nicolson and Johnston 1979, Robinson 1994, Hodge 2003, Wolfe *et al.* 2007). This is supported by evidence from St. John, Coleman, and Reid (1983) that AM extramatrical mycelium preferentially associate with decomposing OM. A further consideration is that soil OM (SOM) is itself a heterogeneous mixture consisting of plant, animal and microbial materials in all stages of decay. There may be multiple compartments or nutrient pools (active, slow and passive) depending upon rate of decomposition (Metherell *et al*. 1993). Thus, the

turnover of these components varies continuously. Fungi are the main producers of litter-degrading enzymes (Yadav and Malanson 2007), and Schneider *et al*. (2012) describe a saprophytic fungal succession where Mucoromycotina, followed by Ascomycota, decompose cellulose and sugars, and Basidiomycota, with their ability to degrade the recalcitrant lignin-containing litter material, appear only later. Metherell *et al*. (1993) suggest the slow pool may have a turnover-time of 20–50 years, and the passive pool 400–2000 years. The three pools are simultaneously dynamic.

6.1.1. Soil chemistry: Nutrient deficiency, inter-relationships, and interactions

The evidence indicates the majority of nutrients made available to plants are derived from the SOM and silt/clay fractions in the transect soils. Both were deficient, OM recorded (after removal of >2 mm fragments) at 0.025-0.05% in St. 1-6, 1.45% in St.7 (ranging up to 2.1%), and silt/clay fraction around 2% in St. 1-6 and slightly more than 6% in St. 7. Atmospheric N deposition can be significant (Mackey *et al*. 2010) but Goa records in 2011-2012 (Yadav *et al*. 2012) showed negligible input, 1.34 $\pm$ 0.8 mg m^{-3} during 2011 monsoon, aerosol samples during winter 2011-2012 6.14 $\pm$ 2.3 mg m^{-3}. Phosphates derived from Ca (mean 12-14 µg g^{-1}; range 7-20 µg g^{-1}) and Fe (mean 8.36 µg g^{-1} in foredunes) were of limited availability even in the higher pH value foredunes. Phosphate availability is greatest at pH range 6.5-7.0 (Brady 1974). Borggaard *et al.* (1990) suggested iron oxide capacity to adsorb phosphates in sandy soils was limited. Even in St. 7, where concentrations had markedly increased, nutrients over the transect in comparison to recommended agricultural crop-plant sufficiency were limited.

Below pH 6.0 most P_i will be present as monovalent H_2PO_4 species (Schachtman, Reid, and Ayling 1998, Krishnaraj and Alagawadi 2002, Smith and Read 2008). The majority of studies on the pH dependence of P_i uptake in higher plants have found that uptake rates are highest between pH 5.0 and pH 6.0 (Schachtman, Reid, and Ayling 1998), coinciding with St.

4-7 region of the transect, and indicates that P taken up by the direct pathway is primarily the monovalent form (Ullrich-Eberius, Novacky, and van Behl 1984, Furihata, Suzuki, and Sakurai 1992).

The Ch. 3 data indicate, and discussion above suggests, the OM in the system is a nutrient complex. Correlation analysis indicated K_2O and Mg were principally sourced from silt/clay fraction, and P from both OM and silt/clay. Calcium was adsorbed on neither OM nor silt/clay. Nitrogen is sourced from OM, atmospheric deposition and in the St. 7 area from bovine defecation. Mean NH_4-N concentrations in the partial transect dataset St. 4-6 were severely limiting at 0.01%, rising to 0.03% (range up to 0.058%) in St. 7. Kachi and Hirose (1983) suggested that low supply of inorganic nitrogen in a Japan coastal dune system was attributable to restriction of mineralization and nitrification of OM by low water-holding capacity of sand.. Zn chelates into OM (Himes and Barber 1978), and OM is adsorbed on Fe-oxide (Gu *et al*. 1994, 1995). Calcium forms a "bridge" between humic acids and clay aggregates, flocculating OM with clays (Muneer and Oades 1989). Fe- and Al-oxides are also strong flocculants, and can reduce the available surface area for adsorption of OM onto clay particle (Six *et al*. 2002).

There was strong positive correlation indicated between means OM and Bray-extractable P in the transect survey dataset. Interrelations between OM and P are closely associated, Adams and Walker (1975) and Adams, Campbell, and Cutler (1975) finding organic matter accumulation declined as P_o was lost from the system by leaching. McGill and Cole (1981) proposed a conceptual model in soil pedogenesis, supported by extensive data interpretation from the literature, that describes a dichotomy from the previously accepted biological mineralization process which they termed biochemical mineralization. The former mineralizes C and N that have been stabilized together, releasing inorganic forms of N from

organic materials as C is oxidized by soil organisms in the search for energy. The latter mineralizes organic P (P_o) and sulphate esters that have been stabilized independently of the soil organic moiety and mineralized by enzymatic catalysis external to soil organism cells. This suggests Bray's method which is designed to extract adsorbed inorganic (P_i) forms of phosphate only may not detect labile P fraction concentrations. To be available to plants, P_i must be either desorbed or solubilised, and P_o must be mineralised, from pools of total soil P to release orthophosphate, which are the species absorbed by both AM hyphae and plant roots, into the soil solution.

Soil P may be spatially compartmentalized. Metherell *et* al. (1993), in their Century Model, described five mineral pools, labile, two adsorbed regimes with degrees of bond-strength that inter-exchange with labile P, parent P and occluded P. The labile fraction rapidly equilibriates with the soil solution (c.f. Cole, Innis, and Stewart 1977, Smith and Read 2008) and, Metherell *et* al. (1993) suggest, is equivalent to resin extractable P (Zou, Binkley, and Doxtader 1992). The bicarbonate extractable P method (Colwell 1963: see also Tiessen, Stewart, and Cole 1984 for comprehensive list of analytical methods of the various P fractions in soils) originally developed by Olsen *et al.* (1954) for the estimation of plant-available soil P (Turner and Haygarth 2003) also analyses labile P. These analytical methods of labile P may be more useful additional methods of estimating AM-available soil P at fine scale.

The available phosphorus data in the transect survey presents an interesting anomaly where there is considerable fluctuation in availability, at times concentrations high enough to support plant requirement by the direct pathway, even in stations where there is limited resource, and at other times deficient. Spatial heterogeneity of nutrient resource has been referred to above. An extensive literature search has uncovered only one similar temporal

observation where the authors described a fine-scale diurnal fluctuation of Bray-extractable P concentrations in Costa Rica wet tropical forest oxisols (Vandecar *et al*. 2009). The phenomenon should perhaps be investigated further. If it is a common feature in this, or in any soil, it may have considerable significance in AM fungal symbiosis biology, adding a further important temporal population dynamic variable.

The geophysical and chemical features of soils, even in the "simplistic" psammite soils anticipated at the outset of the research, are clearly highly complex. If the biology of AM nutrient function at fine scale in the field is to be clarified, soil analysis should be undertaken at a finer scale than was conducted in this study.

6.1.2. Comparisons of AM spore datasets

Comparison is made of AM spore abundance and distribution over the transect between sample collections pre- and post-monsoon from Ch. 3, and vermicompost amended from Ch. 5. Correlation analysis at genus level is shown in Table 6.1. The table indicates significant correlation between *Acaulospora* post-monsoon (4.12.11), and *Acaulospora* vermicompost amended treatment distributions, between four of the six datasets in Gigasporaceae, weak correlation between post- and pre- (25.5.12) monsoon, and between amended pots and post-monsoon, and post- and pre-monsoon, in *Glomus*. ANOVA indicates no significant difference between means distribution in *Acaulospora* and *Glomus* along the transect, and significant difference in Gigasporaceae ($F = 0.8353$, $P = 0.045$). Difference between correlated post- and pre-monsoon Gigasporaceae ($t = 1.15$, df = 12, $P = 0.027$) was a significant increase in pre-monsoon abundance in St. 4 and 5, and between pre-monsoon and amended pots ($t = 1.07$, df = 12, $P = 0.030$), abundance in St. 1 and 2 in amended treatment

decreasing significantly. *Acaulospora* abundance increased over the dry season, principally in St. 1 and 2. *Acaulospora* levels in amended pots were less than half of those in both the post-

Table 6.1. Pearson's correlation coefficient of AM spore distribution at genus level over the transect.

	r	*P*
Acaul. amend : *Acaul*. 4.12.11	0.887	0.004
Acaul. amend : *Acaul*. 25.5.12	0.383	n/s
Acaul. 4.12.11 : *Acaul.* 25.5 12	0.472	n/s
*Acaul.*control *: Acaul.* Amend	0.054	n/s
*Acaul.*control : *Acaul.* 4.12.11	-0.085	n/s
*Acaul.*control : *Acaul.* 25.5.12	-0.049	n/s
Gig. amend : Gig. 4.12.11	0.689	0.040
Gig. amend : Gig. 25.5.12	0.747	0.030
Gig. 4.12.11 : Gig. 25.5.12	0.671	0.050
Gig. control : Gig. amend	0.652	n/s
Gig. control : Gig. 4.12.11	0.949	0.001
Gig. Control : Gig. 25.5.12	0.437	n/s
Gl. amend : *Gl.* 4.12.11	0.882	0.004
Gl. amend : *Gl.* 25.5.12	0.367	n/s
Gl. 4.12.11 : *Gl.* 25.5.12	0.689	0.040
*Gl.*control : *Gl.* amend	0.679	n/s
Gl. control : *Gl.* 4.12.11	0.420	n/s
Gl. control : *Gl.* 25.5.12	-0.185	n/s

25.5.12 = pre-monsoon, 4.12.11 = post monsoon, amend = *I. indicum* vermicompost amendment treatment, control = vermicompost control.

and pre-monsoon sets. Whether this was a direct effect of vermicompost addition cannot be ascertained. It may be an indication of increase in sporulation rate in response to increased plant diversity (Burrows and Pfleger 2002), post- and pre-monsoon samples having been extracted from rhizospheres of all plants present in each station. Gigasporaceae distribution remained fairly constant over the different treatments, again increasing in abundance during the dry season, and again decreasing in single plant rhizosphere despite vermicompost amendment. The highly significant control : 4.12.11 correlation shows little variation in

abundance in stations over the transect. As *Glomus* abundance is comparatively very low the genus is discussed no further.

It was suggested functionality, and particularly nutrient-function efficiency, may be highlighted by compiling and comparing fine-scale, species level spore datasets. Heterogeneity, however, may be a fundamental confounding factor in fine-scale analysis. Tews and Koske (1986), for example, found up to 30 samples were needed to ensure recovery of all species present in sand dune soil. In the current study a minimum of 20 sub-samples, 5 x 4 plant species^{-1} station^{-1} in the transect studies, amplified by five different occasions in the Ch. 3 study, would suggest the strategy was adequate. In old-field ecosystems, AM fungi can be spatially patchy in both abundance (Boerner, DeMars, and Leicht 1996) and composition (Hart and Klironomos 2002, Pringle and Bever 2002) at small spatial scales. It is also probable that, in competing for scarce patchy resource, sporulation occurs in discrete heterogenous clusters in soil that, at fine-scale, may not have been representatively recovered despite sampling 'north-south-east-west' rhizosphere soils. On one occasion only, for example, *Gi. rosea* Nicol. & Schenck (Plate 2.2c) spores were surprisingly recovered from a single sub-sample, surprising as this is believed to be the first report of the species in India west-coast dunes. At fine-scale spore diversity and abundance would seem chaotic, pattern emerging only at a broader scale (Taylor *et al*. 2002, Cavender-Bares *et al*. 2009, Verbruggen *et al*. 2012).

Transect-wide linear correlation of spore abundance at species level indicates highly significant similarities (Table 6.2) in all stations prior to the humic soils of St. 7. Yet another anomaly in St. 5 further suggests there may be fundamental variance in AM fungal biology at this location that is worthy of further investigation. A finer scale analysis, in this instance,

Table 6.2. Pearsons correlation coefficient at species level.

	amend : 25.5.12		amend : control		control : 25.5.12	
	r	*P*	r	*P*	r	*P*
St. 1	0.939	<0.001	0.853	<0.001	0.907	<0.001
St. 2	0.952	<0.001	0.358	0.033	0.493	0.005
St. 3	0.625	<0.001	0.938	<0.001	0.603	<0.001
St. 4	0.864	<0.001	0.765	<0.001	0.861	<0.001
St. 5	0.196	0.154*	0.504	0.004	0.160	0.204*
St. 6	0.742	<0.001	0.681	<0.001	0.731	<0.001
St. 7	0.179	0.176*	0.326	0.049	0.021	0.457*
Total	0.761	<0.001	0.902	<0.001	0.812	<0.001

amend = vermicompost amended (Ch. 5 study), control = vermicompost control (Ch. 5 study), 25.5.12 = pre-monsoon (Ch. 3 survey). (n = 27).
* = non-significance.

seems to indicate pattern. Where there is significant correlation in all stations between amended and control, at genus level (Table 5.3) correlation was indicated in stations 1 and 3 , and overall, only. The results do support the hypothesis of overall AM fungal structural, and hence functional, stability in the ecosystem however, despite, or perhaps as a result of (May and Oster 1976, May 1986, Levin 1992), abundance and diversity variation at fine-scale. Fitter *et al*. (2005) suggest soils appear able to retain function even when their biological structure has been radically altered.

A further example of variation in representation of data at coarse and fine scales is shown in scatterplot comparison of Simpson's AM spore diversity at genus- and species-level against plant frequency (Fig. 6.1). The species-level plot highlights the trended increase in diversity

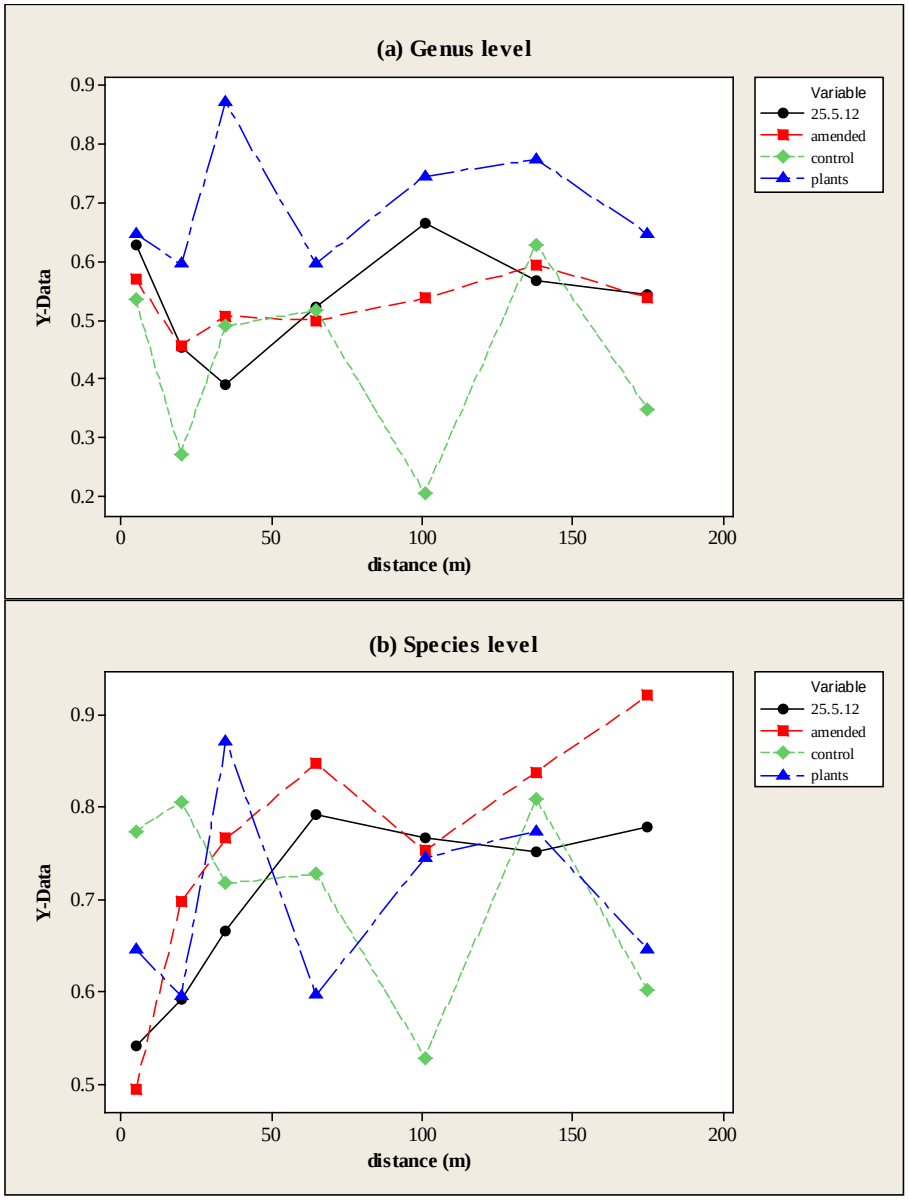


Fig. 6.1. Scatterplots of Simpson's diversity along the transect of plant frequency at species level, with AM spore diversity at genus (**a**) and species (**b**) levels.

described in Figure 5.11 that is not evident at genus level. Similarly, and interestingly, the species-level plot shows a steep rise in AM spore diversity in pre-monsoon (25.5.12) data up to St. 4, followed by a distinct plateau to St. 7 that indicates ecosystem stability that is usually associated with increasing diversity (Tilman, Lehman, and Bristow 1998, Tilman, Reich, and Knops 2000).

Correspondence analysis of AM spore abundance and diversity data at species level in the three treatments pre-monsoon (25.5.12), vermicompost amendment, and control, over distance along the transect is shown in Figure 6.2. The scatterplot shows clear pattern along the *x* axis (distance). All three treatments at 175 m (St. 7), the humic soils region on the

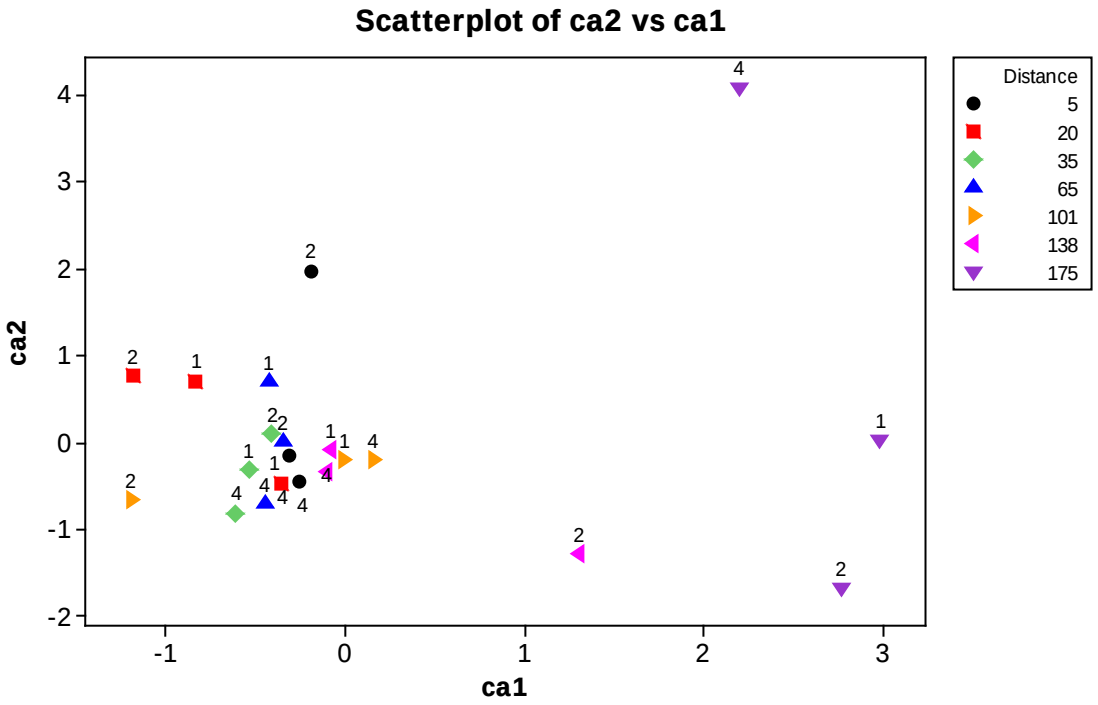


Fig. 6.2. Correspondance analysis of amended pots (treatment **1**), control pots (treatment **2**) and pre-monsoon (treatment **4**) (ca2) spore distribution against station distance (ca1) over the transect. The two axes explain 40.3% and 34.3% (total 74.6%) of the variation respectively.

transect where there is a marked reduction in pH and distinct shift in AM spores abundance and species diversity described in Ch. 3 and 5, stand at a discete distance. The AM pathway is not entirely excluded at low pH however. The presence of spores indicates some AM mycelial activity, supported by evidence of plant root colonization that ranges from 8% to a high 25% in *I. indicum* early growth stages.Three of the remaining six control datapoints (St. 1, 2 and 5) show variability between treatments, St. 6 affected by distance. The remaining

datapoints are tightly clustered indicating high similarity in transect distribution, again indicating structural stability.

Comparison is made (Fig. 6.3) of spore abundance in the four dominant species highlighted in Ch. 5 Discussion (*A. spinosa*, *A. scrobiculata*, *Gi. margarita* and *S. gregaria*) between the three species-level datasets, pre-monsoon 25.5.12 (together 76.3% of total spores), Ch. 5 vermicompost amended (79.4%), and vermicompost control (71.6%).

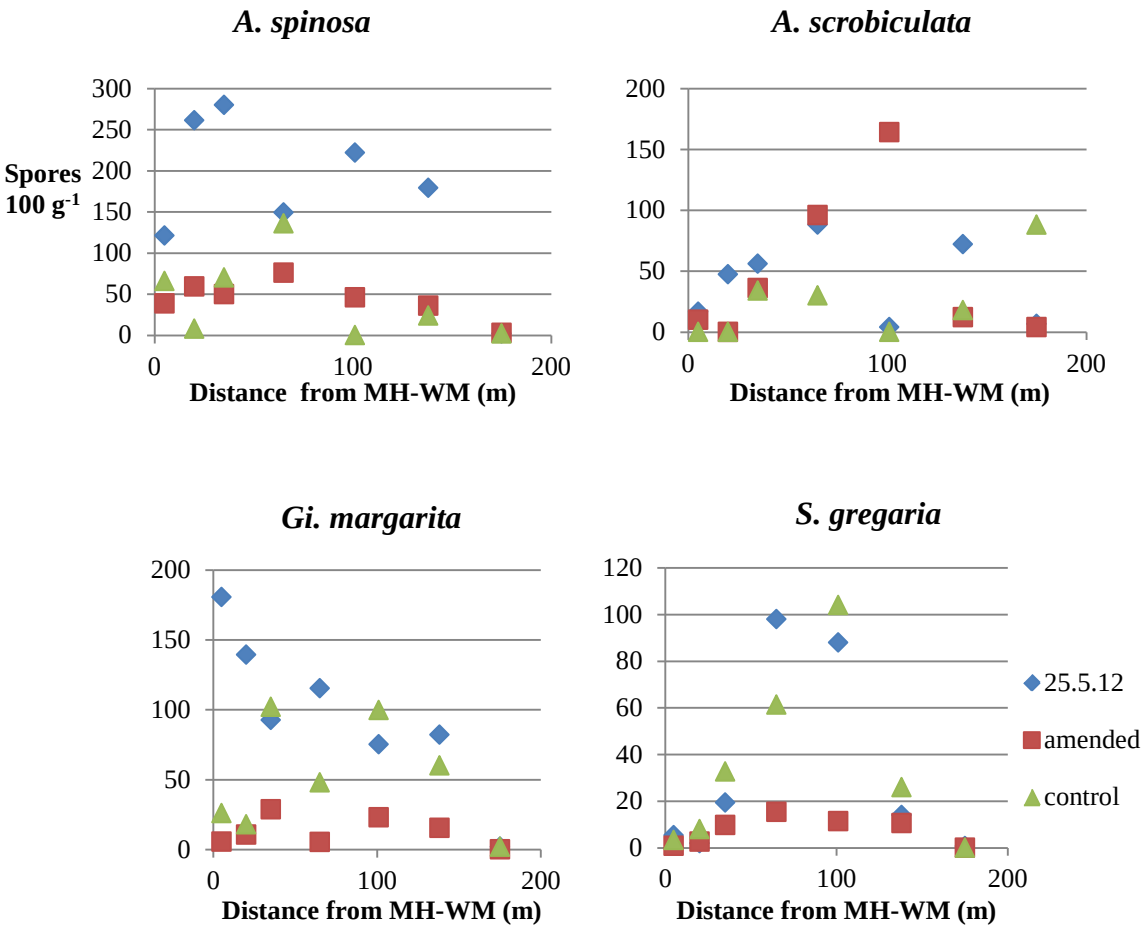


Fig. 6.3. Distribution of AM species that are commonly co-dominant in pre-monsoon (25.5.12), vermicompost amended and vermicompost control.

Pearson's analysis of distribution of each species indicates significant correlation between 25.5.12 (pre-monsoon) and vermicompost amended *A. spinosa* transect-wide spores (r =

0.687; *P* = 0.044), no significant correlation in *A. scrobiculata*, and significant correlation in amended : control *Gi. margarita* (r = 0.794; *P* = 0.016). There was significant correlation in two of three *S. gregaria* comparisons, r = 0.923 (*P* = <0.002) in pre-monsoon : amended and r = 0.884 (*P* = 0.004) in pre-monsoon : control. There was weak correlation between amended and control (r = 0.663; *P* = 0.052). Similarity of all three treatments in *S. gregaria* suggests an element of functional stability in the species, but analysis does not indicate there might be similarity in nutrient function.

Transect-wide correlation analysis applied inter-specifically revealed a highly significant relationship between *A. scrobiculata* and *S. gregaria* in amended treatments (r = 0.980; *P* = <0.0001). Abundance in *A. scrobiculata* was considerably greater however, suggesting contribution to nutrient facilitation may have been greater. Significant correlation was also indicated between *Gi. margarita* and *A. spinosa* in pre-monsoon data (r = 0.778; *P* = <0.020), here abundance in *A. spinosa* greater except in St. 1. There was also significant correlation between *Gi. margarita* and *S. gregaria* in amended treatments (r = 0.734; *P* = 0.030).

These results reinforce the evidence for overall AM fungal functional stability, and imply there may be order found in data retrieved at fine-scale despite the difficulties referred to with small-scale heterogeneity data generation. There seems little intra-specific relatedness between them, however, except for correlations in *S. gregaria*. Furthermore, they give little or no indication of nutrient-function efficiency.

ANOVA of the four species in each station (Table 6.3) indicates *A. spinosa* is significantly greater in abundance in all stations but St. 7 in pre-monsoon (T1) data. Interpretation of the constancy is not clear-cut. It suggests the species is unaffected by additional OM in vermicompost amendment, a total of 1066 spores recovered over the transect from T1, 309

Table 6.3. ANOVA of the four AM species in **Fig. 6.3** comparing abundance in 3 treatments at each station.

			F	*P*
St. 1	AM 1	sig. greater in T1	7.45	0.024
	AM 2	ditto	5.79	0.040
	AM 3	ditto	134.71	<0.001
	AM 4	n/s		
St. 2	AM 1	sig. greater in T1	157.23	<0.001
	AM 2	ditto	51.97	<0.001
	AM 3	ditto	29.47	0.001
	AM 4	sig. greater in T2&T3	6.75	0.029
St. 3	AM 1	sig. greater in T1	24.50	0.001
	AM 2	n/s		
	AM 3	n/s		
	AM 4	n/s		
St. 4	AM 1	sig. greater in T1&T3	6.85	0.028
	AM 2	n/s		
	AM 3	sig. greater in T1	16.81	0.003
	AM 4	n/s		
St. 5	AM 1	sig. greater in T1	23.30	0.001
	AM 2	sig. greater in T2	14.35	0.005
	AM 3	n/s		
	AM 4	n/s		
St. 6	AM 1	sig. greater in T1	40.37	<0.001
	AM 2	ditto		
	AM 3	n/s		
	AM 4	n/s		
St. 7	AM 1	n/s		
	AM 2	sig. greater in T3	1538.80	<0.001
	AM 3	n/s		
	AM 4	n/s		

AM 1 = *A. spinosa*
AM 2 = *A. scrobiculata*
AM 3 = *Gi.margarita*
AM 4 = *S. gregaria*

T1= 25.5.12
T2 = vermicompost amended
T3 = vermicompost control

spores from T2. Neither is there parity between T1 and control soils (T3) that represent the natural soil, 304 spores recovered, a similar figure to that in T2, which reinforces a nul-effect interpretation. However, as described above, greater T1 abundance may be a response to

spore recovery from multiple plant species (Burrows and Pfleger 2002). *Acaulospora scrobiculata* (AM2) was also significantly greater in abundance in T1 St. 1 and 2, significantly greater in the anomalous St. 5 in amended, highly significantly greater in St. 7 T3 where 88 spores were recovered as against 4 in amended and 7 in T1. Over the transect 241 spores were recovered from T1, 312 from T2, and 170 from T3. *Gigaspora margarita* was the second-most abundant species recovered from T1, 687 spores, the most abundant in T2 (356 spores), and also in T3 (422). ANOVA indicates *Gi. margarita* abundance was significantly greater in T1 in St. 1, St. 2, and St. 4. No other significant relationship is indicated. Again the statistic suggests there was little vermicompost amendment influence on the species. *Scutellaspora gregaria* was significantly greater only in T2 and T3 in St. 2 but density was low. Total abundances in T1 and T2 were equivalent, 234 and 235 spores respectively, suggesting comparative recovery from the single host plant species indicates an amendment effect.

6.1.3. Two nutrients, two AM species.

Which of the macro-nutrients N and P might be the principal limiting factor (Sprengel-Liebig's Law of the minimum: van der Ploeg, Bo¨hm, and Kirkham 1999) in the dune system cannot be conclusively ascertained. Soils, and particularly agricultural soils, are often described as P-deficient, even where >90% of P is adsorbed (e.g. Richardson *et al*. 2009). Discussion of the relevance of P_i : $P_{o,}$ and different methods of analysis has been offered above. It is not unreasonable to suggest the estimates made of P concentration in the study were lower than total plant- and AM-available P where labile-P had not been accounted for. In deficient soil AM are particularly efficient in P-nutrient facilitation. Nitrogen may have been the limiting factor when plants climbed onto dry land, and soil OM accumulation may have been a long process. An aggrading sand-dune system pedogenesis process is analogous.

Slowly advancing newly-generated fore-dunes require plant-invasion to stabilize structurally, chemically, and biotrophically. Organic matter accumulation increases over space, the primary-dune sand left behind the advancing fore-dune, and time. Again discussion of OM chemistry has been offered above. It is now evident that AM fungi play as important a role in NH_4-N, NO_3 (Bago *et al*. 1996, Govindarajulu *et al*. 2005, Jin *et al*. 2005) and amino-acid sequestration and transport (Hawkins, Johansen, and George 2000) as that in P nutrient-function, and plant N-requirement is far greater in volume (Hodge, Helgason, and Fitter 2010). The soil analyses data in Ch. 3 and 4 suggest N is the more deficient here, despite the very low P_2O_5 concentrations in *S. littoreus* rhizosphere soils in the foredunes.

Two AM species have proved consistently dominant in all species-level soil sample analyses, *A. spinosa* and *Gi. margarita*. Table 6.4 lists spore abundance of the two species

Table 6.4. Abundance of 2 co-dominant AM spores in vermicompost treatments.

A. spinosa				*Gi. margarita*			
distance	amended	control	sachet	distance	amended	control	sachet
5	39	66	2	5	26	70	8
20	59	8	0	20	18	34	2
35	50	70	0	35	102	166	194
65	76	136	2	65	48	30	46
101	46	0	0	101	100	90	74
138	36	24	0	138	60	32	20
175	3	2	8	175	2	0	58
total	309	304	12		356	422	402

recovered in the Ch. 5 vermicompost amendment experiment. The strategies of the two species differ. ANOVA of *A. spinosa* over the transect, excluding the reduced St. 7 humic soil abundance, shows there are no significant differences ($F = 3.01$; $P = 0.055$) between stations, supporting the ‘generalist’ strategy proposed above. A similar ANOVA of *Gi.*

margarita indicates significant reduction in St. 1 and 2 (F = 7.45: *P* = 0.002). A further, and striking, strategic variance is negligible *A. spinosa* abundance in residual vermicompost sachets compared with greater than amended pots abundance of *Gi. margarita*, substantiated by St. 3 root breach. Douds and Schenck (1990), in an elegant nutrient-manipulation pot experiment using the tropical bahia grass (*Paspalum notatum* Flugge) in sandy soil low in N and available P, found increase in N concentrations increased *Gi. margarita* sporulation.

The differentiation strongly implies at least one of the species may be particularly associated with one of the nutrients N and P. CCA in the transect survey had indicated significant correlation of *A. spinosa* with OM, and non-significant association with P_2O_5, suggesting the taxon may have been preferentially scavenging N nutrient. It is reported that in N-acquisition, where plant roots and AM hyphae penetrate the same patch of OM, mycelium development is reduced (Hodge *et al*. 2000, 2003). This would appear to be a plausible plant strategy. Why contribute hard-earned C to a symbiotic organism in exchange for a nutrient that can be readily taken up *via* the direct pathway? Root exclusion from vermicompost must then encourage AM mycelium penetration, here predominantly *Gi. margarita*, but not necessarily in exclusive pursuit of N. Veresoglou, Shaw and Sen (2011), comparing nutrient facilitation by *G. intraradices* and *Gi. margarita*, again in nutrient deficient sand-dune soils, found N limitation in tissues of the host plant *Plantago lanceolata* L. in a *Gi. margarita* treatment. The data recorded here do not substantiate which of the two AM species is efficiently capturing which nutrient.

Except for vermicompost amendment effect on *Gi. albida* indicated in ANOVA, there is no direct evidence to support the Thesis hypothesis. On the premise that spore abundance is an indication of nutrient function efficiency, none of the most abundant AM taxa encountered is statistically proven to be predominantly, and consistently, coupled with a specific nutrient.

Robustness of data and their interpretation is lastly discussed as there are a number of assumptions, anomalies and experimental flaws that may have had gross effect. It is unwise to draw emphatic conclusion of ecological features in the study site without data from subsequent annual cycles, more than a single transect line, and also perhaps from other dune systems where similar plant zonation pattern is evident, to ascertain constancy. Thus, and coupled with a flawed data-retrieval programme, i.e. no complete set in either edaphic or environmental data, nor spores data, over the three year study period, those drawn should be treated with reservation. Further, a principal assumption made in the Introduction that an empirical record of AM spores at genus and/or species level may be analogous to nutrient-function may also be suspect. The literature suggests there is no constancy in the relationship between sporulation and root distribution (Friese and Koske 1991, Olsson, Jakobson, and Wallander 2002), nor may there be between sporulation and mycelium density or functional activity (Zhao *et al*. 2001). Spore spatial and temporal distribution and density may also vary in response to long- and short-term environmental variation (Gemma and Koske 1988, Escudero and Mendoza 2005, Ehinger, Koch, and Sanders 2009). St. John and Koske (1988) suggest AM fungal spores may occur in clumped distributions in the field, and thus the study sampling strategy may be at risk. Furthermore, comparison between molecular and morphological survey has detected AM fungal species that may not sporulate or species that sporulate at a different time of the year from the time of sampling in field studies (Clapp *et al*. 2002).

Nevertheless spore abundance analysis has illuminated a number of ecological features, traditional and novel, in a west coast India coastal dune system. The study has proved complex. It has described a little of the wondrous complexity of AM fungal ecology, and partly illustrated the nutrient-function role of the organism in a primary coastal sand-dune

ecosystem, that is but one of the many roles played. It has described a remarkable cohesion and stability in ecosystem structure that may be directly related to the AM fungal community. It has highlighted the importance of investigation of interactions between plants, soil chemistry characteristics, environment, and trophic levels of soil organisms in natural ecosystems that might be applied to conservation, restoration or sustainable agriculture. It has given a tantalizing glimpse into the fascinating world of soil, and provoked many unanswered questions.

6.2. Conclusions

- The dune system is aggrading but incipient dune is prone to washout by the intensity of the India west-coast monsoon.
- Plant foliar biomass is controlled by wind-shear and wind-blown sand particles. There is a zonation pattern in plant demography along the transect. Density is associated with a reducing pH gradient.
- Plant community composition and AM community composition are spatially structured at different scales.
- The first transition zone in the dune sytem is below-ground, a pedo-ecotone, where a humus content in the sand matrix became evident. This is reflected in decreased pH, increased nutrient status, and increased plant density. Plants remain prostrate.
- All edaphic features are variable and temporal fluctuation in soil P availability may have significant effect upon AM fungal dynamic.
- Conversely, correlation and CA indicate AM fungal structure facilitates ecosystem stability where edaphic and environmental factors are spatially and temporally variable.
- Coarse- (AM genus level) and fine- (AM species level) scale analyses render varying data interpretation.
- *Glomus* taxa are significantly less abundant throughout the transect, increasing to become dominant in St. 7.
- *A. spinosa* and *Gi. margarita* are co-dominant in the transect up to St. 7 and display dissimilar strategies.
- There is evidence to suggest AM fungal sequestration of N may have more significant ecosystem function implication than sequestration of P.
- There is no evidence to indicate AM taxon specific nutrient-function efficiency.

6.3. Recommendations for Further Work

One novel AM species spore has been extracted from the transect soils and preparation of a manuscript for publication submission describing the genetic sequence and morphology is ongoing (Appendix 1.4). Other possibly novel species have also been unearthed (e.g. Plate 7). All have been only very occasionally and sporadically sighted which may suggest rarity in AM fungi occurs, and perhaps, as has been indicated by comparison with other west-coast India surveys, geographical distribution is limited. None has yet been investigated at the molecular level.

Plate 6.2. Potential novel species spores. a) A specimen that appears to retain sporocarp integrity but has the morphological characteristics of a *Gigaspora* species; b-d) An unidentified *Glomus* specimen extracted from a St. 5 sachet during the vermicompost amendment in *I. indica* study.

The study site is one of the very few coastal sand dune systems not de-spoiled by beach-shack development in Goa. Resource and further effort, if only for the sake of posterity should the study site soon fall foul of ever expanding tourism, ought to be implemented in order to catalogue these and any other novel species that might yet be uncovered. It is recommended that this important work should be undertaken as soon as possible.

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Glossary

Direct and indirect pathways … the direct water and nutrient uptake pathway through root hairs and epidermis, and the indirect AM pathway in which water and nutrient absorbed by soil inhabiting fungal mycelium are translocated to specialized exchange structures (arbuscules) within root cortex cells.

Cover … a method of sampling plant frequency in a given area by the amount of ground the foliage covers, usually by a non-grid quadrat (i.e. not sub-divided into smaller squares) survey. Percentage cover is the area of the replicated quadrats occupied by each species when viewed from above (Greig-Smith 1983, pg. 10).

Driver (driven) … a concept in ecology that describes a causal relationship between organisms (e.g. myxomatosis and rabbit decimation), between an organism and environment (e.g. rainfall and plant growth), or between environmental factors (e.g. CO_2 emission and climate change). Hart, Reader, and Klironomos (2001) proposed a "driver/passenger" concept in AM fungi, an hypothesis where AM fungi are directly controlled by plant diversity, or *vice versa*. The fungi may also be driven by environment e.g. soil chemistry.

Fitness … the outcome of genetic mutations favourable to greater survival of an organism in fluctuating conditions of an environment, a fundamental premise of natural selection and evolution. The fittest species may equate to the dominant species.

Genet … the 'mother' plant of ramets.

Keystone … A keystone species is a species that has a disproportionately large effect on its environment relative to its abundance (Paine 1966, 1995). The species play a critical role in maintaining the structure of an ecological community, affecting many other organisms in an ecosystem and helping to determine the types and numbers of various other species in the community.

MIP … mycorrhizal inoculum potential, the number of viable AM propagules (spores, extraradicle hyphae, intraradicle vesicles) that might further colonize plant roots. Spores are the principle source of MIP in the dune system due to desiccation of plant roots and AM mycelium in the hot dry season between monsoons.

Noise … the effect of chaotic and near chaotic ecological dynamics, temporal and spatial fine-scale changes in the ecosystem, that can obscure the line of determinism. Soil biota spatial heterogeneity, for example, has been described as noise (Ettema and Wardle 2002). Controlled experiments reduce the number of inter-active parameters e.g. soil sterilization excludes microbial effects.

Plant rooted frequency … a method of sampling plant frequency in a given area by counting the number of rooted individuals, usually by replicated quadrat (sub-divided into a grid of smaller squares) survey. Species are counted where rooted in each quadrat sub-division (Greig-Smith 1983, Pg. 10). This was the method used in the transect survey in the study, 16 x 100 mm^2 sub-divided squares x 5 replicates in each station.

Ramet … Prof. John Harper's "singular contribution to the Oxford English Dictionary" (Undergrad. lecture 1982, University College of North Wales, Bangor): any of the individuals in a group of clones.

Recalcitrant … molecular-level characteristics of organic substances, including elemental composition and molecular conformation, that influence OM resistance to degradation by microbial organisms and enzymes.

Scarp … where the seaward-facing dune has been cut away by high seas leaving a vertical face (Plate 3.2, Pg. 65). The height depends upon how far back into the sloping dune the water has cut.

Stability … little deviation in an ecosystem from its average state (resistance) or the capability of an ecosystem to return to its original state following a disturbance or other

perturbation (resilience). There is evidence that biodiversity increases the stability of ecosystem processes in changing environments (Loreau and de Mazancourt 2013). In dune systems primary dunes are described as unstable and inland forest as stable (Olson 1958).

Appendices

Appendix 1: Papers published and in preparation:

Appendix 1.1. Radhika, K.P., Willis, A., and Rodrigues, B.F. (2009) 'Emergence of germ tube from germination shield in *Scutellospora verrucosa* Walker & Sanders' *Indian Journal of Mycology and Plant Pathology* 39: 317-319

Emergence of Germ Tube from Germination Shield in *Scutellospora verrucosa* Walker & Sanders

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Abstract

A germ tube emergence from the germ tube initial of germination shield was recorded in a spore of *S. verrucosa* isolated from *Neragamia alata* W & A (Meliaceae). The germ tube was found to be present in the inner germinal walls of the spore and it penetrated the outer wall. The germ tube emergence from germination shield can be used as a marker to determine the systematic position of *Scutellospora* genera.

Key words- Germ tube**,** *S. verrucosa*, germination shield, germ tube initial

Citation: Radhika KP, Willis A and Rodrigues BF. 2009. Emergence of germ tube from germination shield in *Scutellospora verrucosa* Walker & Sanders. *J Mycol Pl Pathol* 39(2):317-319.

Appendix 1.2. Willis, A. and Rodrigues, B.F. (2009) 'Polymerase Chain Reaction (PCR) in Arbuscular Mycorrhizal (AM) Research' In *Arbuscular Mycorrhizae of Goa – Manual of Identification Protocols.* ed. by Rodrigues, B. F. and Muthukumar, T. Goa: Goa University

Arbuscular Mycorrhizae of Goa - A Manual of Identification Protocols

Editors: B. F. Rodrigues & T. Muthukumar

POLYMERASE CHAIN REACTION (PCR) IN ARBUSCULAR MYCORRHIZA (AM) RESEARCH

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Appendix 1.3. Willis, A., Rodrigues, B.F., and Harris, P.J.C. (2013) 'The ecology of arbuscular mycorrhizal fungi' *Critical Reviews in Plant Sciences* 32: 1-20

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TheEcologyofArbuscularMycorrhizalFungi

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Appendix 1.4. Willis, A., Vaingankar, J.D., Adholeya, A., and Harris, P.J.C. (**in preparation**) '*Glomus felinovii*, a novel arbuscular mycorrhizal fungal species from coastal sand dunes in Goa, India' (as at 15.4.13)

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MYCOTAXON style 2012 **November 1, 2011, revision**

Glomus felinovii, a novel arbuscular mycorrhizal fungal species from coastal sand dunes in Goa, India

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ABSTRACT — During a survey on a coastal dune system in Goa, India, a previously undescribed Glomeromycota spore was recovered. The species produces white to golden-yellow to dull brown spores with a distinctive integral funnel-shaped appendage on the surface, quite unlike any other species described in the phylum. Detailed morphological characteristics are described. Molecular analysis places the novel species in *Glomus* genus and the fungus is described as *Glomus felinovii* sp. nov.

KEY WORDS — Glomerales, species description, spore morphology, taxonomy, molecular phylogeny